JC10 Rec'd PCT/PTO 13 DEC 2001

| FORM PTO-1390 U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE (MODIFIED)<br>**TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371** | ATTORNEY'S DOCKET NUMBER<br>**X-11260**<br>U.S. APPLICATION NO. (if known, see 37 C.F.R. 1.5)<br>10/018214 |
|---|---|

| INTERNATIONAL APPLICATION NO. | INTERNATIONAL FILING DATE | PRIORITY DATE CLAIMED |
|---|---|---|
| **PCT/US00/15017** | **08 June 2000 (08.06.00)** | **15 July 1999 (15.07.99)** |

TITLE OF INVENTION: **PSEUDOMYCIN N-ACYL SIDE-CHAIN ANALOGS**

APPLICANT(S) FOR DO/EO/US: **Matthew David Belvo, Shu Hui Chen, Christopher William Doecke, Sarah Lynne Hellman, James Andrew Jamison, Lawrence Edward Patterson, Michael John Rodriguez, Xicheng David Sun, William Wilson Turner, Venkatraghavan Vasudevan, and Mark James Zweifel**

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [ ] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. [X] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [ ] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] has been transmitted by the International Bureau.
   c. [X] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [ ] A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. [X] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been transmitted by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [X] have not been made and will not be made.
8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [X] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [X] A copy of the International Preliminary Examination Report (IPER), including any annexes, and, if not in English, an English language translation of the annexes to the IPER under PCT Article 36 (35 U.S.C. 371(c)(5)).

**Items 11. to 16. below concern document(s) or information included:**

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [X] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [X] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [ ] Other items or information:

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| U.S. APPLICATION NO. (if known, see 37 C.F.R. 1.5) | INTERNATIONAL APPLICATION NO. | ATTORNEY'S DOCKET NUMBER |
|---|---|---|
| 10/018214 | PCT/US00/15017 | X-11260 |

17. [X] The following fees are submitted:

| | CALCULATIONS | PTO USE ONLY |
|---|---|---|
| **BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):**<br>Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO. **$1040.00**<br><br>International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO ............ **$890.00**<br><br>International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO............ **$740.00**<br><br>International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) ..... **$710.00**<br><br>International preliminary examination fee paid to USPTO (37 CRF 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) ........**$100.00** | | |
| **ENTER APPROPRIATE BASIC FEE AMOUNT =** | $ 890.00 | |
| Surcharge of **$130.00** for furnishing the oath or declaration later than __20 __30 months from the earliest claimed priority date (37 CFR 1.492(e)). | $ | |

| CLAIMS | NUMBER FILED | NUMBER EXTRA | RATE | | |
|---|---|---|---|---|---|
| Total claims | 15 -20= | 0 | **X $18.00** | $ | |
| Independent claims | 4 - 3= | 1 | **X $84.00** | $ 84.00 | |
| MULTIPLE DEPENDENT CLAIM(S) (if applicable) | | | **+ $280.00** | $ | |

| | | |
|---|---|---|
| **TOTAL OF ABOVE CALCULATIONS =** | $ 84.00 | |
| Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28). | $ | |
| **SUBTOTAL =** | $ 974.00 | |
| Processing fee of **$130.00** for furnishing English translation later than __ 20 __30 months from the earliest claimed priority date (37 CFR 1.492(f)). + | $ | |
| **TOTAL NATIONAL FEE =** | $ 974.00 | |
| Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property | $ | |
| **TOTAL FEES ENCLOSED =** | $ 974.00 | |
| | **Amount to be refunded** | $ |
| | **charged** | $ |

a. [ ] A check in the amount of $_________ to cover the above fees is enclosed.

b. [X] Please charge my Deposit Account No. 05-0840 in the amount of $ 974.00 to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 05-0840. A duplicate copy of this sheet is enclosed.

**NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.**

SEND ALL CORRESPONDENCE TO:
**ELI LILLY AND COMPANY**
PATENT DIVISION/1104
LILLY CORPORATE CENTER
INDIANAPOLIS, INDIANA 46285

25885
PATENT TRADEMARK OFFICE

Tina M. Tucker
SIGNATURE

**Tina M. Tucker**
NAME

**47,145**
REGISTRATION NUMBER

**(317) 277-3537**
TELEPHONE NUMBER

13 Dec 2001
Date

10/018214

JC07 Rec'd PCT/PTO 13 DEC 2001

"Express Mail" mailing label number EL 230530064 US

Date of Deposit Dec. 13, 2001

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

Queen Thomas / Printed Name

Queen Thomas / Signature

**PATENT APPLICATION**
**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

| | | |
|---|---|---|
| Applicant(s): | Belvo, et al. | |
| Serial No.: | Unknown | |
| Filed: | June 8, 2000 | Group Art Unit: Unknown |
| For: | Pseudomycin N-Acyl Side-Chain Analogs | Examiner: Unknown |
| Docket No.: | X-11260 | |

**Preliminary Amendment**

Assistant Commissioner for Patents
Washington, D. C. 20231

Sir:

Applicants submit the following preliminary amendments and remarks in connection with the filing of the above-identified application.

Please amend the application as follows:

**In the Specification**

On page 4 of the specification, after the sentence, "$R^c$ is hydrogen, hydroxy, $C_1$-$C_4$ alkoxy...;" please insert the following

--$R^d$ is hydrogen;--

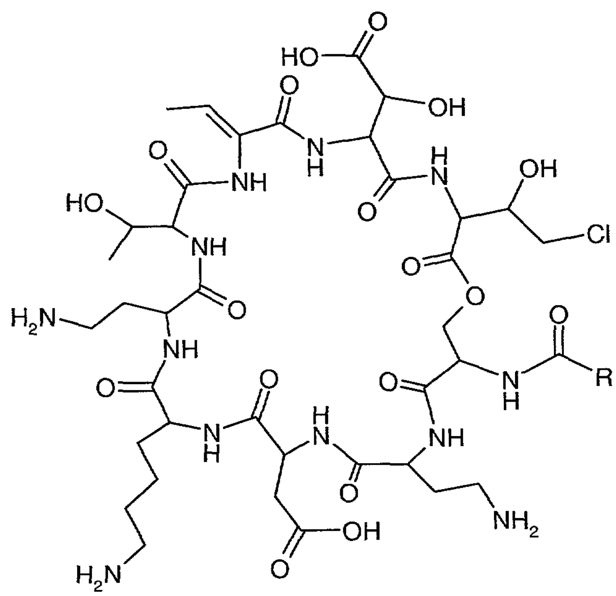
On page 6 of the specification, line 8, please replace "an antifungal" with "a fungal".

## In the Claims

Please cancel Claim 5 without prejudice or disclaimer of any of the subject matter contained herein.

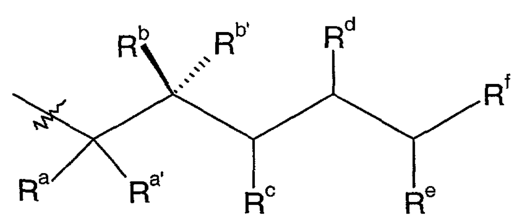
Please amend the claims as follows:

1. (Amended) A compound represented by structure I



I

wherein R is



,

where

$R^a$ and $R^{a'}$ are independently hydrogen or methyl, or either $R^a$ or $R^{a'}$ is alkyl amino, taken together with $R^b$ or $R^{b'}$ forms a six-membered cycloalkyl ring, a six-membered aromatic ring or a double bond, or taken together with $R^c$ forms a six-membered aromatic ring;

$R^b$ and $R^{b'}$ are independently hydrogen, halogen, or methyl, or either $R^b$ or $R^{b'}$ is amino, alkylamino, α-acetoacetate, methoxy, or hydroxy provided that $R^{b'}$ is not

hydroxy when $R^a$, $R^b$, $R^d$, $R^e$ are hydrogen, $R^c$ is hydrogen and $R^f$ is *n*-hexyl, *n*-octyl or *n*-decyl, or $R^a$, $R^b$, $R^d$, $R^e$ are hydrogen, $R^c$ is hydroxy and $R^f$ is *n*-octyl, *n*-nonyl, or *n*-decyl;

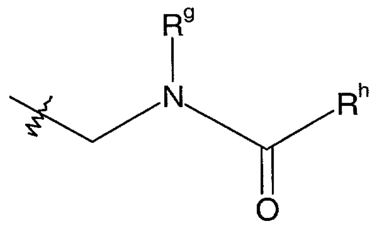
$R^c$ is hydrogen, hydroxy, $C_1$-$C_4$ alkoxy, hydroxyalkoxy, or taken together with $R^e$ forms a 6-membered aromatic ring or $C_5$-$C_6$ cycloalkyl ring;

$R^d$ is hydrogen;

$R^e$ is hydrogen, or taken together with $R^f$ is a six-membered aromatic ring, $C_5$-$C_{14}$ alkoxy substituted six-membered aromatic ring, or $C_5$-$C_{14}$ alkyl substituted six-membered aromatic ring, and

$R^f$ is $C_8$-$C_{18}$ alkyl, $C_5$-$C_{11}$ alkoxy, or biphenyl; or

R is



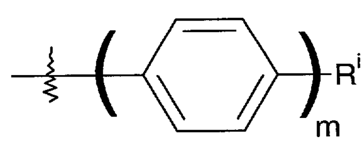

,

where

$R^g$ is hydrogen, or $C_1$-$C_{13}$ alkyl, and

$R^h$ is $C_1$-$C_{15}$ alkyl, $C_4$-$C_{15}$ alkoxy, ($C_1$-$C_{10}$ alkyl)phenyl, -$(CH_2)_n$-aryl, or -$(CH_2)_n$-($C_5$-$C_6$ cycloalkyl), where n = 1-2; or

R is



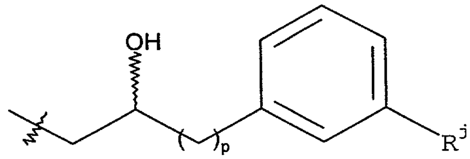

,

where

$R^i$ is a hydrogen, halogen, or $C_5$-$C_8$ alkoxy, and

m is 1, 2 or 3;

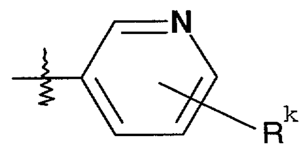
R is




where

$R^j$ is $C_5$-$C_{14}$ alkoxy or $C_5$-$C_{14}$ alkyl, and p = 0, 1 or 2;

R is



where

$R^k$ is $C_5$-$C_{14}$ alkoxy; or

R is -($CH_2$)-$NR^m$-($C_{13}$-$C_{18}$ alkyl), where $R^m$ is H, -$CH_3$ or –C(O)$CH_3$; and

pharmaceutically acceptable salts and solvates thereof.

6. (Amended) A pharmaceutical formulation comprising said pseudomycin compound or said pharmaceutically acceptable salt or solvate thereof of Claim 2 and a pharmaceutically acceptable carrier, diluent, buffer, or excipient.

7. (Amended) A method for treating a fungal infection in an animal in need thereof, comprising the steps of administering to said animal said pseudomycin compound or said pharmaceutically acceptable salt or solvate thereof of Claim 2.

Please add the following new claims:

--16. A pharmaceutical formulation comprising said pseudomycin compound or said pharmaceutically acceptable salt or solvate thereof of Claim 1 and a pharmaceutically acceptable carrier, diluent, buffer, or excipient.--

--17. A method for treating a fungal infection in an animal in need thereof, comprising the steps of administering to said animal said pseudomycin compound or said pharmaceutically acceptable salt or solvate thereof of Claim 1.--

## REMARKS

Claim 5 has been canceled. Claims 1 and 6-7 have been amended. Claims 16 and 17 have been newly added. Basis for this new claims can be found on page 6, lines 3-10. Thus, claims 1-4 and 6-17 are presently in the application.

In regard to claim 1, the variable "$R^d$" was included in the definition of "R", but the definition of $R^d$, itself, was inadvertently omitted. However, support for amending claim 1 to include a definition of $R^d$ can be found in the definition of "$R^c$" in claim 1 as originally filed. Inasmuch as $R^c$ and $R^e$ may form a six-membered aromatic ring, $R^d$, thus, must at least be hydrogen. As such, $R^d$ in claim 1 and the specification at page 4 has been amended to recite hydrogen.

In regard to claims 6-7, these claims have been amended to include a pharmaceutically acceptable salt or solvate thereof, a buffer, a diluent or a excipient in the formulation. Basis for the amendments can be found in claim 1 and on page 24, lines 18-25, to page 25, lines 1-4. Additionally, claims 6-7 have been amended to correct the antecedent basis ("a" has been replaced by "said"). Furthermore, claim 7 has been amended to correct an obvious typographical error ("an antifungal" infection has been replaced by "a fungal" infection). Basis for this amendment can be found on page 26, lines 5-23.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned **"<u>Version with markings to show changes made.</u>"**

For the Examiner's convenience, a clean claim set is attached.

Early and favorable action on the merits is respectfully requested.

Please charge any fees or credit any overpayment in connection with this application which may be required by this or any related paper to Deposit Account No. 05-0840.

If the Examiner has any questions, or would like to discuss any matters in connection with this application, he or she is invited to contact the undersigned at (317) 277-3537.

Respectfully submitted,

Tina M. Tucker

Tina M. Tucker
Agent for Applicants
Registration No. 47,145
Phone: 317-277-3537

Eli Lilly and Company
Patent Division/TMT
Lilly Corporate Center
Indianapolis, Indiana 46285

16 Nov 2001

Attachments: Clean Claim Set

## VERSION WITH MARKINGS TO SHOW CHANGES MADE

### In the Specification

On page 4 of the specification, after the sentence, "$R^c$ is hydrogen, hydroxy, $C_1$-$C_4$ alkoxy...;" please insert the following

--$R^d$ is hydrogen;--

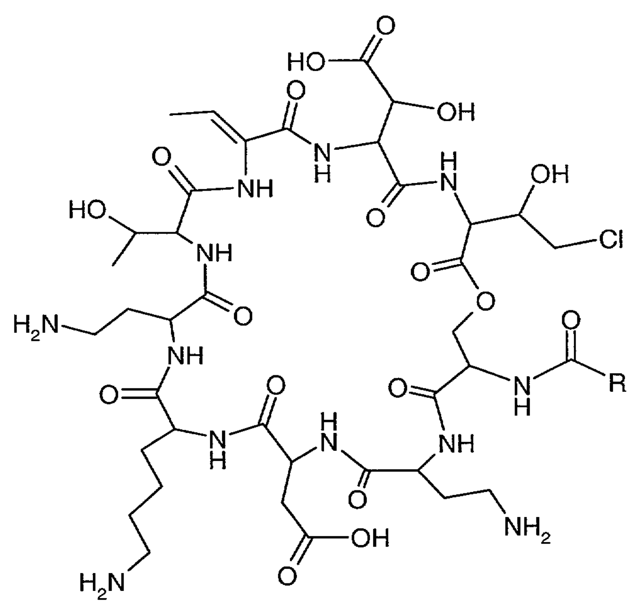
On page 6 of the specification, line 8, please replace "an antifungal" with "a fungal".

### In the claims:

Claim 5 has been cancelled.

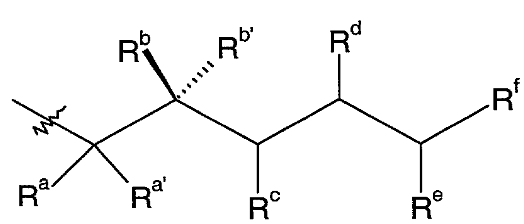
The claims have been amended as follows:

1. (Amended) A compound represented by structure I



I

wherein R is



,

where

$R^a$ and $R^{a'}$ are independently hydrogen or methyl, or either $R^a$ or $R^{a'}$ is alkyl amino, taken together with $R^b$ or $R^{b'}$ forms a six-membered cycloalkyl ring, a six-membered aromatic ring or a double bond, or taken together with $R^c$ forms a six-membered aromatic ring;

$R^b$ and $R^{b'}$ are independently hydrogen, halogen, or methyl, or either $R^b$ or $R^{b'}$ is amino, alkylamino, α-acetoacetate, methoxy, or hydroxy provided that $R^{b'}$ is not hydroxy when $R^a$, $R^b$, $R^d$, $R^e$ are hydrogen, $R^c$ is hydrogen and $R^f$ is *n*-hexyl, *n*-octyl or *n*-decyl, or $R^a$, $R^b$, $R^d$, $R^e$ are hydrogen, $R^c$ is hydroxy and $R^f$ is *n*-octyl, *n*-nonyl, or *n*-decyl;

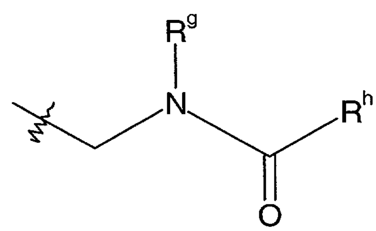
$R^c$ is hydrogen, hydroxy, $C_1$-$C_4$ alkoxy, hydroxyalkoxy, or taken together with $R^e$ forms a 6-membered aromatic ring or $C_5$-$C_6$ cycloalkyl ring;

$R^d$ is hydrogen;

$R^e$ is hydrogen, or taken together with $R^f$ is a six-membered aromatic ring, $C_5$-$C_{14}$ alkoxy substituted six-membered aromatic ring, or $C_5$-$C_{14}$ alkyl substituted six-membered aromatic ring, and

$R^f$ is $C_8$-$C_{18}$ alkyl, $C_5$-$C_{11}$ alkoxy, or biphenyl; or

R is



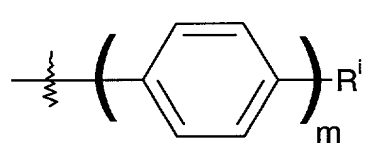
,

where

$R^g$ is hydrogen, or $C_1$-$C_{13}$ alkyl, and

$R^h$ is $C_1$-$C_{15}$ alkyl, $C_4$-$C_{15}$ alkoxy, ($C_1$-$C_{10}$ alkyl)phenyl, -$(CH_2)_n$-aryl, or -$(CH_2)_n$-($C_5$-$C_6$ cycloalkyl), where n = 1-2; or

R is



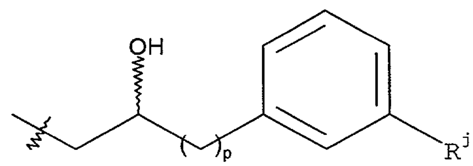
,

where

$R^i$ is a hydrogen, halogen, or $C_5$-$C_8$ alkoxy, and

m is 1, 2 or 3;

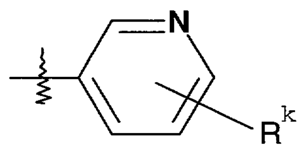
R is



where

$R^j$ is $C_5$-$C_{14}$ alkoxy or $C_5$-$C_{14}$ alkyl, and p = 0, 1 or 2;

R is



where

$R^k$ is $C_5$-$C_{14}$ alkoxy; or

R is -($CH_2$)-$NR^m$-($C_{13}$-$C_{18}$ alkyl), where $R^m$ is H, -$CH_3$ or –C(O)$CH_3$; and

pharmaceutically acceptable salts and solvates thereof.

6. (Amended) A pharmaceutical formulation comprising [a] said pseudomycin compound or said pharmaceutically acceptable salt or solvate thereof of Claim 2 and a pharmaceutically acceptable carrier, diluent, buffer, or excipient.

7. (Amended) A method for treating [an antifungal] a fungal infection in an animal in need thereof, comprising the steps of administering to said animal [a] said pseudomycin compound [of Claim 2] or said pharmaceutically acceptable salt or solvate thereof of Claim2.

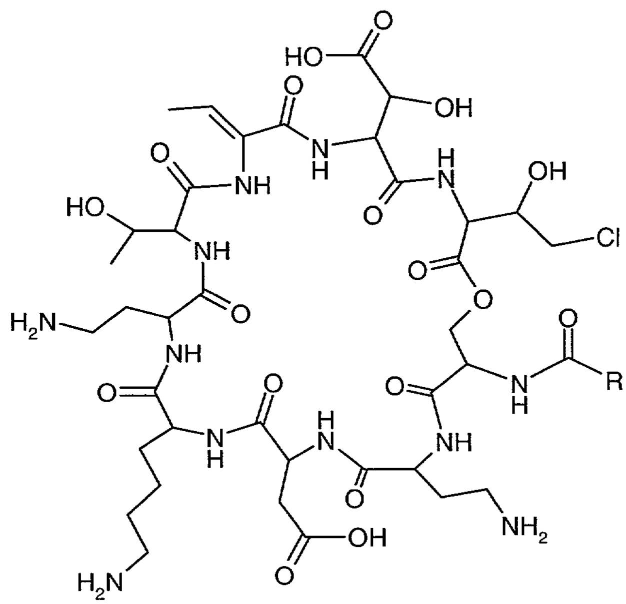
Claims 16 and 17 have been added as follows:

--16. A pharmaceutical formulation comprising said pseudomycin compound or said pharmaceutically acceptable salt or solvate thereof of Claim 1 and a pharmaceutically acceptable carrier, diluent, buffer, or excipient.--

--17. A method for treating a fungal infection in an animal in need thereof, comprising the steps of administering to said animal said pseudomycin compound or said pharmaceutically acceptable salt or solvate thereof of Claim 1.--

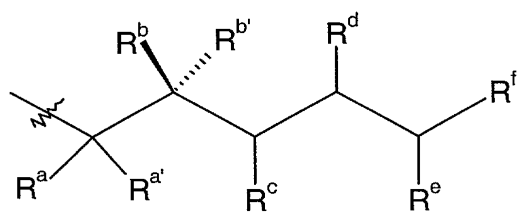
## CLEAN CLAIM SET

1. A compound represented by structure I



I

wherein R is



,

where

$R^a$ and $R^{a'}$ are independently hydrogen or methyl, or either $R^a$ or $R^{a'}$ is alkyl amino, taken together with $R^b$ or $R^{b'}$ forms a six-membered cycloalkyl ring, a six-membered aromatic ring or a double bond, or taken together with $R^c$ forms a six-membered aromatic ring;

$R^b$ and $R^{b'}$ are independently hydrogen, halogen, or methyl, or either $R^b$ or $R^{b'}$ is amino, alkylamino, α-acetoacetate, methoxy, or hydroxy provided that $R^{b'}$ is not hydroxy when $R^a$, $R^b$, $R^d$, $R^e$ are hydrogen, $R^c$ is hydrogen and $R^f$ is *n*-hexyl, *n*-octyl or *n*-decyl, or $R^a$, $R^b$, $R^d$, $R^e$ are hydrogen, $R^c$ is hydroxy and $R^f$ is *n*-octyl, *n*-nonyl, or *n*-decyl;

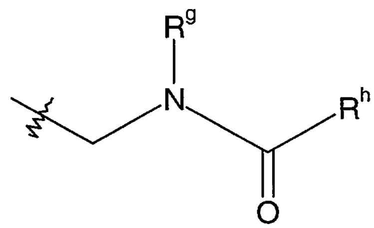
$R^c$ is hydrogen, hydroxy, $C_1$-$C_4$ alkoxy, hydroxyalkoxy, or taken together with $R^e$ forms a 6-membered aromatic ring or $C_5$-$C_6$ cycloalkyl ring;

$R^d$ is hydrogen;

$R^e$ is hydrogen, or taken together with $R^f$ is a six-membered aromatic ring, $C_5$-$C_{14}$ alkoxy substituted six-membered aromatic ring, or $C_5$-$C_{14}$ alkyl substituted six-membered aromatic ring, and

$R^f$ is $C_8$-$C_{18}$ alkyl, $C_5$-$C_{11}$ alkoxy, or biphenyl; or

R is



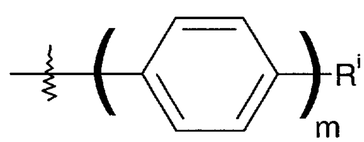
,

where

$R^g$ is hydrogen, or $C_1$-$C_{13}$ alkyl, and

$R^h$ is $C_1$-$C_{15}$ alkyl, $C_4$-$C_{15}$ alkoxy, ($C_1$-$C_{10}$ alkyl)phenyl, -$(CH_2)_n$-aryl, or -$(CH_2)_n$-($C_5$-$C_6$ cycloalkyl), where n = 1-2; or

R is



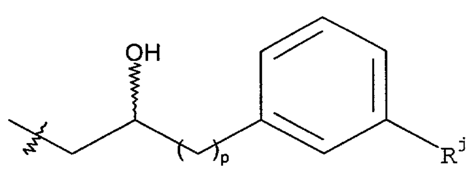
,

where

$R^i$ is a hydrogen, halogen, or $C_5$-$C_8$ alkoxy, and

m is 1, 2 or 3;

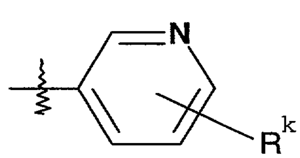
R is



where

$R^j$ is $C_5$-$C_{14}$ alkoxy or $C_5$-$C_{14}$ alkyl, and p = 0, 1 or 2;

R is



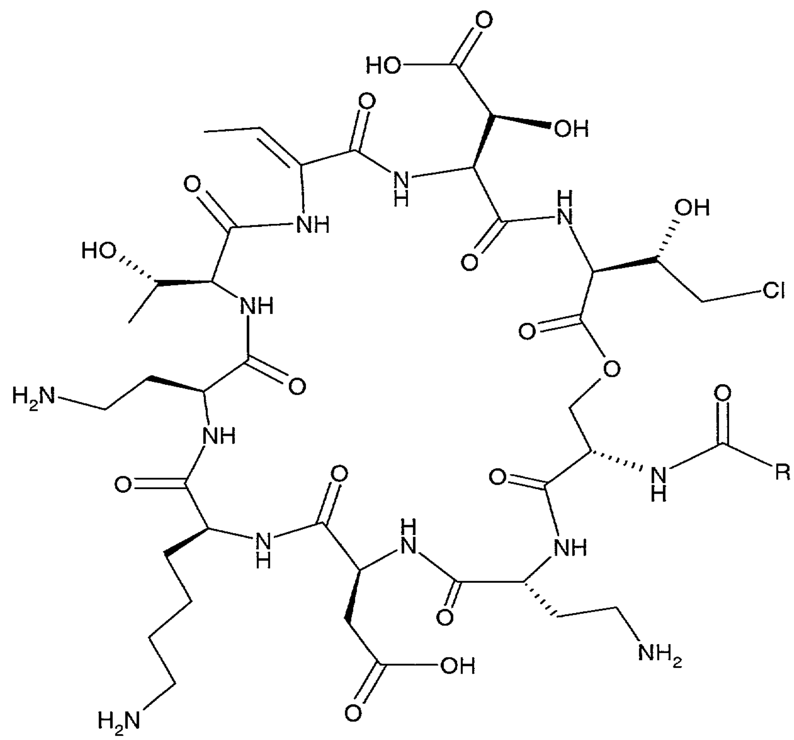
where

$R^k$ is $C_5$-$C_{14}$ alkoxy; or

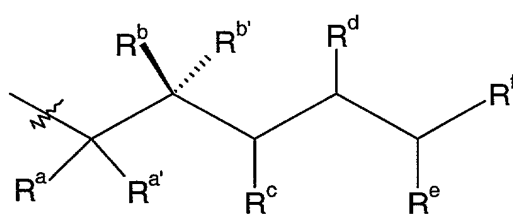
R is -($CH_2$)-$NR^m$-($C_{13}$-$C_{18}$ alkyl), where $R^m$ is H, -$CH_3$ or –C(O)$CH_3$; and

pharmaceutically acceptable salts and solvates thereof.

2. The compound of Claim 1 wherein structure I has the following stereochemistry



3. The compound of Claim 1 wherein R is



,

where

$R^a$ and $R^{a'}$ are independently hydrogen or methyl, or either $R^a$ or $R^{a'}$ is alkyl amino, taken together with $R^b$ or $R^{b'}$ forms a six-membered cycloalkyl ring, a six-

membered aromatic ring or a double bond, or taken together with $R^c$ forms a six-membered aromatic ring;

$R^b$ and $R^{b'}$ are independently hydrogen, halogen, or methyl, or either $R^b$ or $R^{b'}$ is amino, alkylamino, α-acetoacetate, methoxy, or hydroxy provided that $R^{b'}$ is not hydroxy when $R^a$, $R^b$, $R^d$, $R^e$ are hydrogen, $R^c$ is hydrogen and $R^f$ is *n*-hexyl, *n*-octyl or *n*-decyl, or $R^a$, $R^b$, $R^d$, $R^e$ are hydrogen, $R^c$ is hydroxy and $R^f$ is *n*-octyl, *n*-nonyl, or *n*-decyl;

$R^c$ is hydrogen, hydroxy, $C_1$-$C_4$ alkoxy, hydroxyalkoxy, or taken together with $R^e$ forms a 6-membered aromatic ring or $C_5$-$C_6$ cycloalkyl ring;

$R^e$ is hydrogen, or taken together with $R^f$ is a six-membered aromatic ring, $C_5$-$C_{14}$ alkoxy substituted six-membered aromatic ring, or $C_5$-$C_{14}$ alkyl substituted six-membered aromatic ring, and

$R^f$ is $C_8$-$C_{18}$ alkyl, $C_5$-$C_{11}$ alkoxy, or biphenyl.

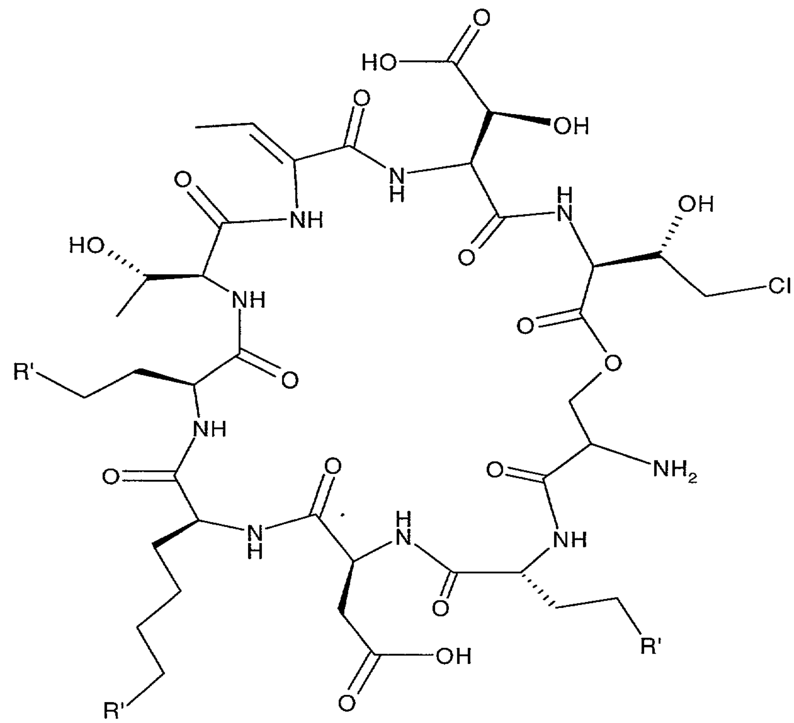
4. The compound of Claim 3 wherein $R^{b'}$ is hydroxy provided that $R^c$ is not hydrogen when $R^a$, $R^b$, $R^d$, $R^e$ are hydrogen and $R^f$ is *n*-hexyl, *n*-octyl or *n*-decyl, or $R^c$ is not hydroxy when $R^f$ is *n*-octyl, *n*-nonyl, or *n*-decyl.

6. A pharmaceutical formulation comprising said pseudomycin compound or said pharmaceutically acceptable salt or solvate thereof of Claim 2 and a pharmaceutically acceptable carrier, diluent, buffer, or excipient.

7. A method for treating a fungal infection in an animal in need thereof, comprising the steps of administering to said animal said pseudomycin compound or said pharmaceutically acceptable salt or solvate thereof of Claim 2.

8. A process for producing a pseudomycin nucleus comprising the steps of providing a pseudomycin compound having an N-acyl alkyl side-chain containing at least one gamma or delta hydroxy group and reacting said pseudomycin compound with an acid to produce said pseudomycin nucleus.

9. The process of Claim 8 wherein said pseudomycin nucleus is represented by structure I-A



**I-A**

wherein R' is $-NH_2$ or -NHp-Pg where Pg is an amino protecting group and p is 0 or 1.

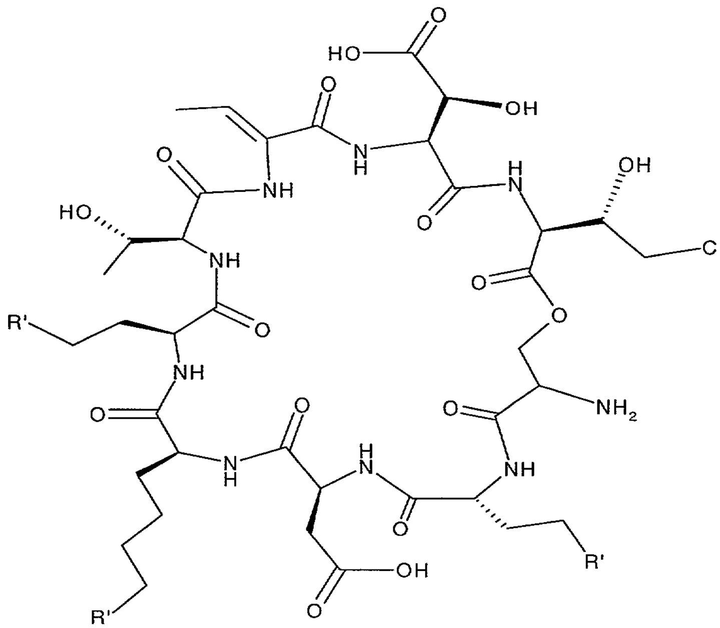
10. The process of Claim 8 wherein said pseudomycin compound having an N-acyl alkyl side-chain containing at least one gamma or delta hydroxy group is selected from the group consisting of pseudomycin A, pseudomycin A' and pseudomycin C.

11. The process of Claim 8 wherein said acid is trifluoroacetic acid or acetic acid.

12. The process of Claim 11 wherein said acid is trifluoroacetic acid.

13. A pseudomycin nucleus prepared by the process of Claim 8.

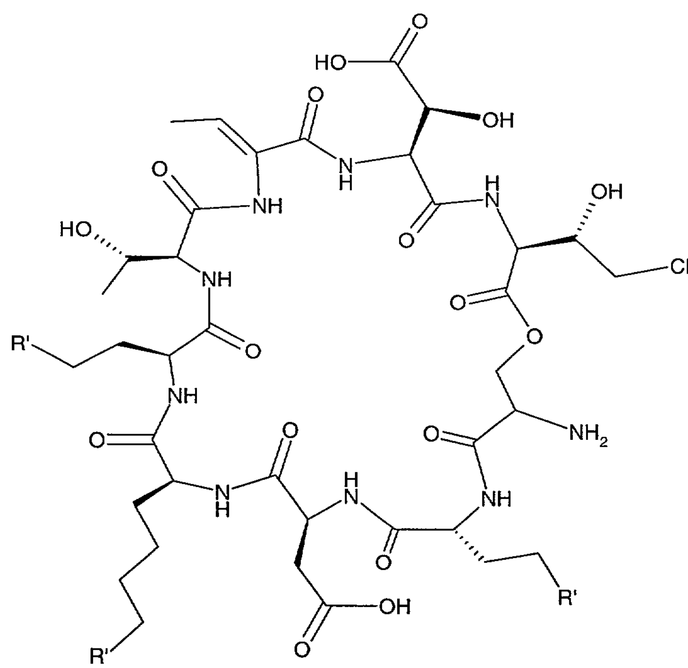
14. The pseudomycin nucleus of Claim 13 wherein said nucleus is represented by structure I-A



**I-A**

wherein R' is $-NH_2$ or -NHp-Pg where Pg is an amino protecting group and p is 0 or 1.

15. A pseudomycin nucleus represented by structure I-A



**I-A**

wherein R' is $-NH_2$ or -NHp-Pg where Pg is an amino protecting group and p is 0 or 1.

16. A pharmaceutical formulation comprising said pseudomycin compound or said pharmaceutically acceptable salt or solvate thereof of Claim 1 and a pharmaceutically acceptable carrier, diluent, buffer, or excipient.

17. A method for treating a fungal infection in an animal in need thereof, comprising the steps of administering to said animal said pseudomycin compound or said pharmaceutically acceptable salt or solvate thereof of Claim 1.

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# PSEUDOMYCIN N-ACYL SIDE-CHAIN ANALOGS

## FIELD OF THE INVENTION

The present invention relates to pseudomycin compounds, in particular, semi-synthetic pseudomycin compounds having novel N-acyl side-chains.

## BACKGROUND OF THE INVENTION

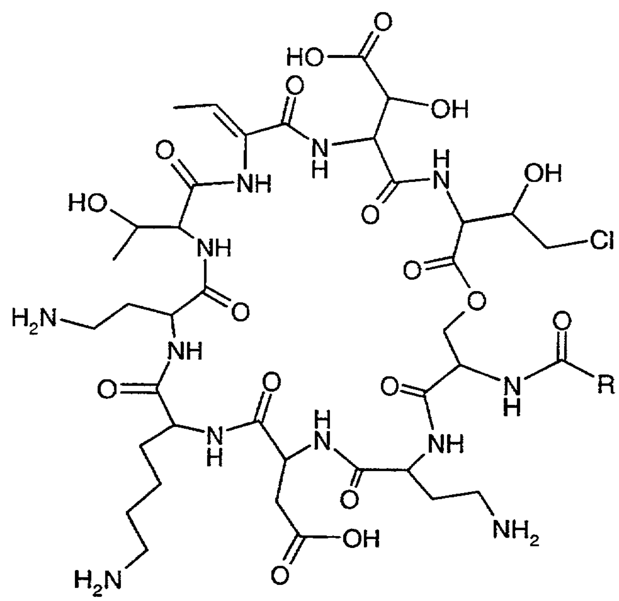
Pseudomycins are natural products isolated from liquid cultures of *Pseudomonas syringae* (plant-associated bacterium) and have been shown to have antifungal activities. (see i.e., Harrison, L., et al., "Pseudomycins, a family of novel peptides from *Pseudomonas syringae* possessing broad-spectrum antifungal activity," J. Gen. Microbiology, **137**(12), 2857-65 (1991) and US Patent Nos. 5,576,298 and 5,837,685) Unlike the previously described antimycotics from *P. syringae* (e.g., syringomycins, syringotoxins and syringostatins), pseudomycins A-C contain hydroxyaspartic acid, aspartic acid, serine, dehydroaminobutyric acid, lysine and diaminobutyric acid. The peptide moiety for pseudomycins A, A', B, B', C, C' corresponds to L-Ser-D-Dab-L-Asp-L-Lys-L-Dab-L-aThr-Z-Dhb-L-Asp(3-OH)-L-Thr(4-Cl) with the terminal carboxyl group closing a macrocyclic ring on the OH group of the N-terminal

Ser. The analogs are distinguished by the N-acyl side chain, i.e., pseudomycin A is N-acylated by 3,4-dihydroxytetradeconoyl, pseudomycin A′ by 3,4-dihydroxypentadecanoyl, pseudomycin B by 3-hydroxytetradecanoyl, pseudomycin B′ by 3-hydroxydodecanoyl, pseudomycin C by 3,4-dihydroxyhexadecanoyl and pseudomycin C′ by 3-hydroxyhexadecanoyl. (see i.e., Ballio, A., et al., "Novel bioactive lipodepsipeptides from *Pseudomonas syringae*: the pseudomycins," FEBS Letters, **355**(1), 96-100, (1994) and Coiro, V.M., et al., "Solution conformation of the *Pseudomonas syringae* MSU 16H phytotoxic lipodepsipeptide Pseudomycin A determined by computer simulations using distance geometry and molecular dynamics from NMR data," Eur. J. Biochem., **257**(2), 449-456 (1998).)

Pseudomycins are known to have certain adverse biological effects. For example, destruction of the endothelium of the vein, destruction of tissue, inflammation, and local toxicity to host tissues have been observed when pseudomycin is administered intraveneously. Therefore, there is a need to identify compounds within this class that are useful for treating fungal infections without the currently observed adverse side effects.

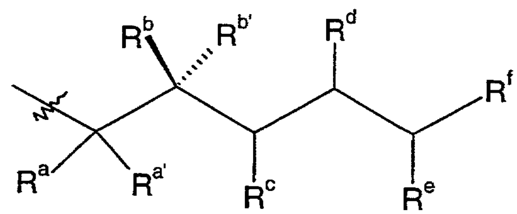
BRIEF SUMMARY OF THE INVENTION

The present invention provides pseudomycin compounds represented by the following structure which are useful as antifungal agents or in the design of antifungal agents.



I

wherein R is



,

where

$R^a$ and $R^{a'}$ are independently hydrogen or methyl, or either $R^a$ or $R^{a'}$ is alkyl amino, taken together with $R^b$ or $R^{b'}$ forms a six-membered cycloalkyl ring, a six-

membered aromatic ring or a double bond, or taken together with $R^c$ forms a six-membered aromatic ring;

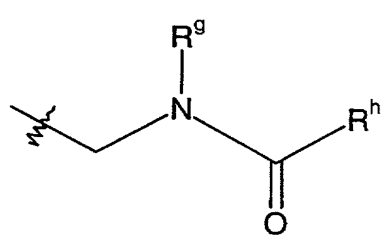
$R^b$ and $R^{b'}$ are independently hydrogen, halogen, or methyl, or either $R^b$ or $R^{b'}$ is amino, alkylamino, α-acetoacetate, methoxy, or hydroxy provided that $R^{b'}$ is not hydroxy when $R^a$, $R^b$, $R^d$, $R^e$ are hydrogen, $R^c$ is hydrogen and $R^f$ is *n*-hexyl, *n*-octyl or *n*-decyl, or $R^a$, $R^b$, $R^d$, $R^e$ are hydrogen, $R^c$ is hydroxy and $R^f$ is *n*-octyl, *n*-nonyl, or *n*-decyl;

$R^c$ is hydrogen, hydroxy, $C_1$-$C_4$ alkoxy, hydroxyalkoxy, or taken together with $R^e$ forms a 6-membered aromatic ring or $C_5$-$C_6$ cycloalkyl ring;

$R^e$ is hydrogen, or taken together with $R^f$ is a six-membered aromatic ring, $C_5$-$C_{14}$ alkoxy substituted six-membered aromatic ring, or $C_5$-$C_{14}$ alkyl substituted six-membered aromatic ring, and

$R^f$ is $C_8$-$C_{18}$ alkyl, $C_5$-$C_{11}$ alkoxy, or biphenyl; or

R is



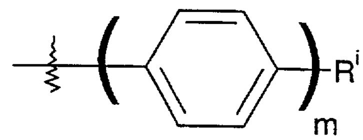

,

where

$R^g$ is hydrogen, or $C_1$-$C_{13}$ alkyl, and

$R^h$ is $C_1$-$C_{15}$ alkyl, $C_4$-$C_{15}$ alkoxy, ($C_1$-$C_{10}$ alkyl)phenyl, -$(CH_2)_n$-aryl, or -$(CH_2)_n$-($C_5$-$C_6$ cycloalkyl), where n = 1 or 2; or

R is

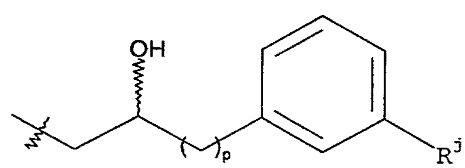


,

where

$R^i$ is a hydrogen, halogen, or $C_5$-$C_8$ alkoxy, and m is 1, 2 or 3;

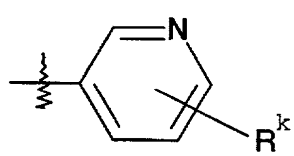
R is



where

$R^j$ is $C_5$-$C_{14}$ alkoxy or $C_5$-$C_{14}$ alkyl, and p = 0, 1 or 2;

R is



where

$R^k$ is $C_5$-$C_{14}$ alkoxy; or

R is -$(CH_2)$-$NR^m$-($C_{13}$-$C_{18}$ alkyl), where $R^m$ is H, -$CH_3$ or

$-C(O)CH_3$; and

pharmaceutically acceptable salts and solvates thereof.

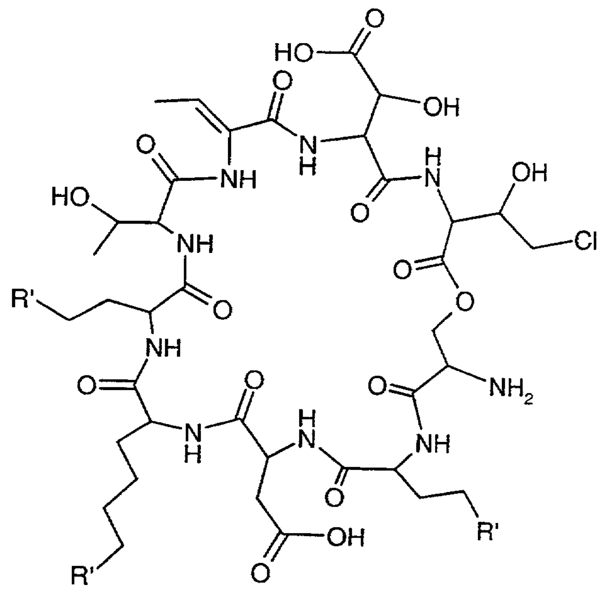
In another embodiment of the present invention, a pharmaceutical formulation is provided which includes the pseudomycin compound represented by structure I above and a pharmaceutically acceptable carrier.

In yet another embodiment of the present invention, a method is provided for treating an antifungal infection in an animal in need thereof, which comprises administering to the animal the pseudomycin compound I described above.

In yet another embodiment of the present invention, a process is provided for producing the free amine nucleus of a pseudomycin compound which may be acylated to form the compounds represented by structure I above. The process includes the steps of treating a pseudomycin compound which contains an N-acyl alkyl side-chain having at least one gamma or delta hydroxyl group (e.g., pseudomycin A, A' or C) with trifluoroacetic acid or acetic acid.

## *Definitions*

As used herein, the term "free amine pseudomycin nucleus" or "pseudomycin nucleus" refers to the structure I-A below:



**I-A**

wherein R′ is $-NH_2$ or -NHp-Pg where Pg is an amino protecting group and p is 0 or 1.

The term "alkyl" refers to a hydrocarbon radical of the general formula $C_nH_{2n+1}$ containing from 1 to 30 carbon atoms unless otherwise indicated. The alkane radical may be straight (e.g. methyl, ethyl, propyl, butyl, etc.), branched (e.g., isopropyl, isobutyl, tertiary butyl, neopentyl, etc.), cyclic (e.g., cyclopropyl, cyclobutyl, cyclopentyl, methylcyclopentyl, cyclohexyl, etc.), or multi-cyclic (e.g., bicyclo[2.2.1]heptane, spiro[2.2]pentane, etc.). The alkane radical may be substituted or unsubstituted. Similarly, the alkyl portion of an alkoxy group, alkanoyl, or alkanoate have the same definition as above.

The term "alkenyl" refers to an acyclic hydrocarbon containing at least one carbon carbon double bond. The alkene radical may be straight, branched, cyclic, or multi-cyclic. The alkene radical may be substituted or unsubstituted. The alkenyl portion of an alkenoxy, alkenoyl or alkenoate group has the same definition as above.

The term "alkynyl" refers to an acyclic hydrocarbon containing at least one carbon carbon triple bond. The alkyne radical may be straight, or branched. The alkyne radical may be substituted or unsubstituted. The alkynyl portion of an alkynoxy, alkynoyl or alkynoate group has the same definition as above.

The term "aryl" refers to aromatic moieties having single (e.g., phenyl) or fused ring systems (e.g., naphthalene, anthracene, phenanthrene, etc.). The aryl groups may be substituted or unsubstituted.

The term "heteroaryl" refers to aromatic moieties containing at least one heteratom within the aromatic ring system (e.g., pyrrole, pyridine, indole, thiophene, furan, benzofuran, imidazole, pyrimidine, purine, benzimidazole, quinoline, etc.). The aromatic moiety may consist of a single or fused ring system. The heteroaryl groups may be substituted or unsubstituted.

"$NH_p$-Pg" and "amino protecting group" refer to a substituent of the amino group commonly employed to block or

protect the amino functionality while reacting other functional groups on the compound. When p is 0, the amino protecting group, when taken with the nitrogen to which it is attached, forms a cyclic imide, *e.g.*, phthalimido and tetrachlorophthalimido. When p is 1, the protecting group, when taken with the nitrogen to which it is attached, can form a carbamate, *e.g.*, methyl, ethyl, and 9-fluorenylmethylcarbamate; or an amide, *e.g.*, N-formyl and N-acetylamide.

Within the field of organic chemistry and particularly within the field of organic biochemistry, it is widely understood that significant substitution of compounds is tolerated or even useful. In the present invention, for example, the term alkyl group allows for substitutents which is a classic alkyl, such as methyl, ethyl, propyl, hexyl, isooctyl, dodecyl, stearyl, etc. The term "group" specifically envisions and allows for substitutions on alkyls which are common in the art, such as hydroxy, halogen, alkoxy, carbonyl, keto, ester, carbamato, etc., as well as including the unsubstituted alkyl moiety. However, it is generally understood by those skilled in the art that the substituents should be selected so as to not adversely affect the pharmacological characteristics of the compound or adversely interfere with the use of the medicament. Suitable substituents for any of the groups defined above

include alkyl, alkenyl, alkynyl, aryl, halo, hydroxy, alkoxy, aryloxy, mercapto, alkylthio, arylthio, mono- and di-alkyl amino, quaternary ammonium salts, aminoalkoxy, hydroxyalkylamino, aminoalkylthio, carbamyl, carbonyl, carboxy, glycolyl, glycyl, hydrazino, guanyl, and combinations thereof.

The term "solvate" refers to an aggregate that comprises one or more molecules of the solute, such as a compound of structure I, with one or more molecules of a pharmaceutical solvent, such as water, ethanol, and the like.

The term "pharmaceutically acceptable salt" refers to organic or inorganic salts of the compounds represented by structure I that are substantially non-toxic to the recipient at the doses administered.

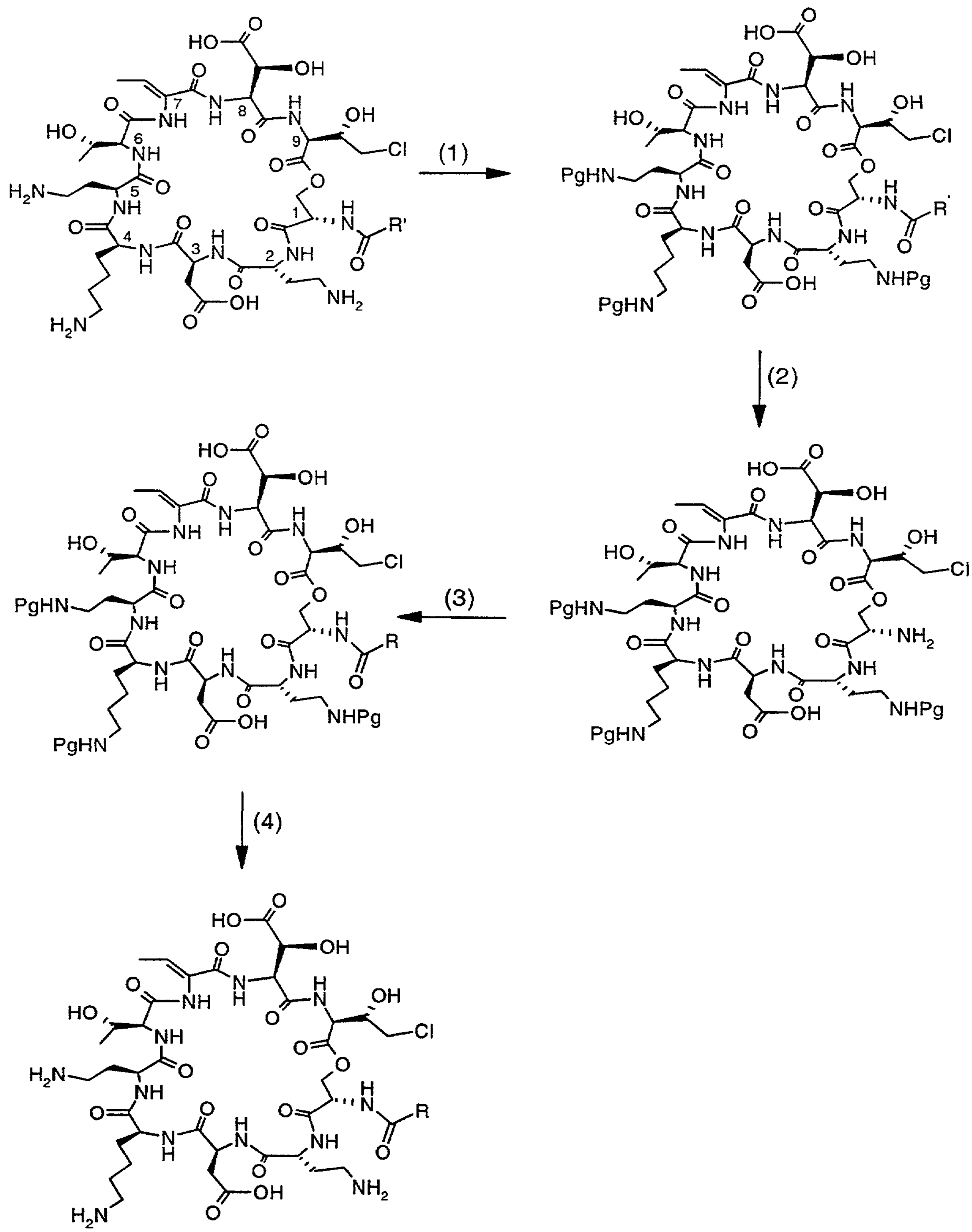
The term "animal" refers to humans, companion animals (e.g., dogs, cats and horses), food-source animals (e.g., cows, pigs, sheep and poultry), zoo animals, marine animals, birds and other similar animal species.

## DETAILED DESCRIPTION OF THE INVENTION

Applicants have discovered that deacylation of the N-acyl group of the L-serine unit of a pseudomycin compound followed by reacylation with a new N-acyl group provides compounds having *in vitro* indications which suggest that the

new compounds may be active against *C. albican, C, neoformans,* and/or *Aspergillus fumigatus.*

Scheme I below illustrates the general procedures for synthesizing Compound I from any one of the naturally occurring pseudomycins. Although a naturally occurring pseudomycin compound is depicted in scheme I, those skilled in the art will understand that side-chain modification of semi-synthetic derivatives of the naturally occurring pseudomycin compounds may be accomplished in a similar manner. In general, four synthetic steps are used to produce Compound I: (1) selective amino protection; (2) chemical or enzymatic deacylation of the N-acyl side-chain; (3) reacylation with a different side-chain; and (4) deprotection of the amino groups.




Scheme I

The pendant amino groups at positions 2, 4 and 5 may be protected using any standard means known to those skilled in the art for amino protection. The exact genus and species of amino protecting group employed is not critical so long as the derivatized amino group is stable to the conditions of subsequent reaction(s) on other positions of the intermediate molecule and the protecting group can be selectively removed at the appropriate point without disrupting the remainder of the molecule including any other amino protecting group(s). Suitable amino-protecting groups include benzyloxycarbonyl, *p*-nitrobenzyloxycarbonyl, *p*-bromobenzyloxycarbonyl, *p*-methoxybenxyloxycarbonyl, *p*-methoxyphenylazobenzyloxycarbonyl, *p*-phenylazobenzyloxycarbonyl, *t*-butyloxycarbonyl, cyclopentyloxycarbonyl, and phthalimido. Preferred amino protecting groups are *t*-butoxycarbonyl (*t*-Boc), allyloxycarbonyl (Alloc), phthalimido, and benzyloxycarbonyl (CbZ or CBZ). Most preferred is allyloxycarbonyl and benzyloxycarbonyl. Further examples of suitable protecting groups are described in T.W. Greene, "Protective Groups in Organic Synthesis," John Wiley and Sons, New York, N.Y., (2nd ed., 1991), at chapter 7.

The deacylation of a N-acyl group having a gamma or delta hydroxylated side chain (e.g., 3,4-dihydroxytetra-deconoate) may be accomplished by treating the amino-

protected pseudomycin compound with a 5-20% aqueous acidic solution. Suitable acids include acetic acid and trifluoroacetic acid. A preferred acid is trifluoroacetic acid. If trifluoroacetic acid is used, the reaction may be accomplished at or near room temperature. However, when acetic acid is used the reaction is generally run at about 40°C. A water soluble organic solvent may be used to assist in solubilizing the pseudomycin compound. Suitable aqueous solvent systems include acetonitrile, water, and mixtures thereof. Acetonitrile was particularly useful when deacylating a protected pseudomycin compound. A preferred acidic solution for deacylating a protected pseudomycin compound is 8% aqueous trifluoroacetic acid in acetonitrile. Organic solvents accelerate the reaction; however, the addition of an organic solvent may lead to other by-products. Pseudomycin compounds lacking a delta hydroxy group on the side chain (e.g., Pseudomycin B and C') may be deacylated enzymatically. Suitable deacylase enzymes include Polymyxin Acylase (164-16081 Fatty Acylase (crude) or 161-16091 Fatty Acylase (pure) available from Wako Pure Chemical Industries, Ltd.), or ECB deacylase (see, e.g., U.S. Patent No. 5,573,936). The enzymatic deacylation may be accomplished using standard deacylation procedures well known to those skilled in the art. For example, general procedures for using Polymyxin acylase may be found in

Yasuda, N., et al, *Agric. Biol. Chem.*, 53, 3245 (1989) and Kimura, Y., et al., *Agric. Biol. Chem.*, 53, 497 (1989).

The deacylated product (also known as the pseudomycin nucleus or "PSN") is reacylated using the corresponding acid of the desired acyl group in the presence of a carbonyl activating agent. "Carbonyl activating group" refers to a substituent of a carbonyl that promotes nucleophilic addition reactions at that carbonyl. Suitable activating substituents are those which have a net electron withdrawing effect on the carbonyl. Such groups include, but are not limited to, alkoxy, aryloxy, nitrogen containing aromatic heterocycles, or amino groups (e.g., oxybenzotriazole, imidazolyl, nitrophenoxy, pentachlorophenoxy, N-oxysuccinimide, N,N'-dicyclohexylisoure-O-yl, and N-hydroxy-N-methoxyamino); acetates; formates; sulfonates (e.g., methanesulfonate, ethanesulfonate, benzenesulfonate, and *p*-tolylsulfonate); and halides (e.g., chloride, bromide, and iodide).

Alternatively, a solid phase synthesis may be used where a hydroxybenzotriazole-resin (HOBt-resin) serves as the coupling agent for the acylation reaction.

A variety of acids may be used in the acylation process. Suitable acids include aliphatic acids containing one or more pendant aryl, alkyl, amino(including primary, secondary and tertiary amines), hydroxy, alkoxy, and amido

groups; aliphatic acids containing nitrogen or oxygen within the aliphatic chain; aromatic acids substituted with alkyl, hydroxy, alkoxy and/or alkyl amino groups; and heteroaromatic acids substituted with alkyl, hydroxy, alkoxy and/or alkyl amino groups. The acylated product may be useful as an active antifungal agent or as an intermediate for the production of an active compound. Even though some compounds were not as useful as others, the activity profiles provide valuable insight into the design trends needed to achieve optimum activity.

Once the amino group is acylated, then the amino protecting groups (at positions 2, 4 and 5) may be removed by hydrogenation in the presence of a hydrogenation catalyst (e.g., 10% Pd/C). When the amino protecting group is allyloxycarbonyl, then the protecting group may be removed using tributyltinhydride and triphenylphosphine palladium dichloride. This particular protection/deprotection scheme has the advantage of reducing the potential for hydrogenating the vinyl group of the Z-Dhb unit of the pseudomycin structure.

As discussed earlier, pseudomycins are natural products isolated from the bacterium *Pseudomonas syringae* that have been characterized as lipodepsinonapetpides containing a cyclic peptide portion closed by a lactone bond and including the unusual amino acids 4-chlorothreonine (ClThr),

3-hydroxyaspartic acid (HOAsp), 2,3-dehydro-2-aminobutyric acid (Dhb), and 2,4-diaminobutyric acid (Dab). Methods for growth of various strains of *P. syringae* to produce the different pseudomycin analogs (A, A', B, B', C, and C') are described below and described in more detail in PCT Patent Application Serial No. PCT/US00/08728 filed by Hilton, et al. on April 14, 2000 entitled "Pseudomycin Production by *Pseudomonas Syringae,*" incorporated herein by reference, PCT Patent Application Serial No. PCT/US00/08727 filed by Kulanthaivel, et al. on April 14, 2000 entitled "Pseudomycin Natural Products," incorporated herein by reference, and U.S. Patent Nos. 5,576,298 and 5,837,685, each of which are incorporated herein by reference.

Isolated strains of *P. syringae* that produce one or more pseudomycins are known in the art. Wild type strain MSU 174 and a mutant of this strain generated by transposon mutagenesis, MSU 16H (ATCC 67028) are described in U.S. Patent Nos. 5,576,298 and 5,837,685; Harrison, et al., "Pseudomycins, a family of novel peptides from *Pseudomonas syringae* possessing broad-spectrum antifungal activity," J. Gen. Microbiology, **137**, 2857-2865 (1991); and Lamb et al., "Transposon mutagenesis and tagging of fluorescent pseudomonas: Antimycotic production is necessary for control

of Dutch elm disease," Proc. Natl. Acad. Sci. USA, **84**, 6447-6451 (1987).

A strain of *P. syringae* that is suitable for production of one or more pseudomycins can be isolated from environmental sources including plants (e.g., barley plants, citrus plants, and lilac plants) as well as, sources such as soil, water, air, and dust. A preferred stain is isolated from plants. Strains of *P. syringae* that are isolated from environmental sources can be referred to as wild type. As used herein, "wild type" refers to a dominant genotype which naturally occurs in the normal population of *P. syringae* (e.g., strains or isolates of *P. syringae* that are found in nature and not produced by laboratory manipulation). Like most organisms, the characteristics of the pseudomycin-producing cultures employed (*P. syringae* strains such as MSU 174, MSU 16H, MSU 206, 25-B1, 7H9-1) are subject to variation. Hence, progeny of these strains (e.g., recombinants, mutants and variants) may be obtained by methods known in the art.

*P. syringae* MSU 16H is publicly available from the American Type Culture Collection, Parklawn Drive, Rockville, MD, USA as Accession No. ATCC 67028. *P. syringae* strains 25-B1, 7H9-1, and 67 H1 were deposited with the American Type Culture Collection on March 23, 2000 and were assigned the following Accession Nos.:

| | |
|---|---|
| 25-B1 | Accession No. PTA-1622 |
| 7H9-1 | Accession No. PTA-1623 |
| 67 H1 | Accession No. PTA-1621 |

Mutant strains of *P. syringae* are also suitable for production of one or more pseudomycins. As used herein, "mutant" refers to a sudden heritable change in the phenotype of a strain, which can be spontaneous or induced by known mutagenic agents, such as radiation (e.g., ultraviolet radiation or x-rays), chemical mutagens (e.g., ethyl methanesulfonate (EMS), diepoxyoctane, N-methyl-N-nitro-N'-nitrosoguanine (NTG), and nitrous acid), site-specific mutagenesis, and transposon mediated mutagenesis. Pseudomycin-producing mutants of *P. syringae* can be produced by treating the bacteria with an amount of a mutagenic agent effective to produce mutants that overproduce one or more pseudomycins, that produce one pseudomycin (e.g., pseudomycin B) in excess over other pseudomycins, or that produce one or more pseudomycins under advantageous growth conditions. While the type and amount of mutagenic agent to be used can vary, a preferred method is to serially dilute NTG to levels ranging from 1 to 100 μg/ml. Preferred mutants are those that overproduce pseudomycin B and grow in minimal defined media.

Environmental isolates, mutant strains, and other desirable strains of *P. syringae* can be subjected to

selection for desirable traits of growth habit, growth medium nutrient source, carbon source, growth conditions, amino acid requirements, and the like. Preferably, a pseudomycin producing strain of *P. syringae* is selected for growth on minimal defined medium such as N21 medium and/or for production of one or more pseudomycins at levels greater than about 10 μg/ml. Preferred strains exhibit the characteristic of producing one or more pseudomycins when grown on a medium including three or fewer amino acids and optionally, either a lipid, a potato product or combination thereof.

Recombinant strains can be developed by transforming the *P. syringae* strains, using procedures known in the art. Through the use of recombinant DNA technology, the *P. syringae* strains can be transformed to express a variety of gene products in addition to the antibiotics these strains produce. For example, one can modify the strains to introduce multiple copies of the endogenous pseudomycin-biosynthesis genes to achieve greater pseudomycin yield.

To produce one or more pseudomycins from a wild type or mutant strain of P. syringae, the organism is cultured with agitation in an aqueous nutrient medium including an effective amount of three or fewer amino acids, preferably glutamic acid, glycine, histidine, or a combination thereof.

Alternatively, glycine is combined with one or more of a potato product and a lipid. Culturing is conducted under conditions effective for growth of *P. syringae* and production of the desired pseudomycin or pseudomycins. Effective conditions include temperatures from about 22ºC to about 27ºC, and a duration of about 36 hours to about 96 hours. Controlling the concentration of oxygen in the medium during culturing of *P. syringae* is advantageous for production of a pseudomycin. Preferably, oxygen levels are maintained at about 5 to 50% saturation, more preferably about 30% saturation. Sparging with air, pure oxygen, or gas mixtures including oxygen can regulate the concentration of oxygen in the medium.

Controlling the pH of the medium during culturing *of P. syringae* is also advantageous. Pseudomycins are labile at basic pH, and significant degradation can occur if the pH of the culture medium is above about 6 for more than about 12 hours. Preferably, the pH of the culture medium is maintained between 6 and 4. *P. syringae* can produce one or more pseudomycins when grown in batch culture. However, fed-bath or semi-continuous feed of glucose and optionally, an acid or base (e.g., ammonium hydroxide) to control pH, enhances production. Pseudomycin production can be further enhanced by using continuous culture methods in which glucose and ammonium hydroxide are fed automatically.

Choice of *P. syringae* strain can affect the amount and distribution of pseudomycin or pseudomycins produced. For example, strains MSU 16H and 67 H1 each produce predominantly pseudomycin A, but also produce pseudomycin B and C, typically in ratios of 4:2:1. Strain 67 H1 typically produces levels of pseudomycins about three to five fold larger than are produced by strain MSU 16H. Compared to strains MSU 16H and 67 H1, strain 25-B1 produces more pseudomycin B and less pseudomycin C. Strain 7H9-1 are distinctive in producing predominantly pseudomycin B and larger amount of pseudomycin B than other strains. For example, this strain can produce pseudomycin B in at least a ten fold excess over either pseudomycin A or C.

Each pseudomycin, pseudomycin intermediate and mixtures can be detected, determined, isolated, and/or purified by any variety of methods known to those skilled in the art. For example, the level of pseudomycin activity in a broth or in an isolate or purified composition can be determined by antifungal action against a fungus such as Candida and can be isolated and purified by high performance liquid chromatography.

The pseudomycin compound may be isolated and used per se or in the form of its pharmaceutically acceptable salt or solvate. The term "pharmaceutically acceptable salt" refers to non-toxic acid addition salts derived from inorganic and

organic acids. Suitable salt derivatives include halides, thiocyanates, sulfates, bisulfates, sulfites, bisulfites, arylsulfonates, alkylsulfates, phosphonates, monohydrogen-phosphates, dihydrogenphosphates, metaphosphates, pyrophosphonates, alkanoates, cycloalkylalkanoates, arylalkonates, adipates, alginates, aspartates, benzoates, fumarates, glucoheptanoates, glycerophosphates, lactates, maleates, nicotinates, oxalates, palmitates, pectinates, picrates, pivalates, succinates, tartarates, citrates, camphorates, camphorsulfonates, digluconates, trifluoroacetates, and the like.

The term "solvate" refers to an aggregate that comprises one or more molecules of the solute (i.e., pseudomycin prodrug compound) with one or more molecules of a pharmaceutical solvent, such as water, ethanol, and the like. When the solvent is water, then the aggregate is referred to as a hydrate. Solvates are generally formed by dissolving the compound in the appropriate solvent with heat and slowing cooling to generate an amorphous or crystalline solvate form.

The active ingredient (i.e., pseudomycin derivative) is typically formulated into pharmaceutical dosage forms to provide an easily controllable dosage of the drug and to give the physician, patient, or veterinarian an elegant and easily handleable product. Formulations may comprise from

0.1% to 99.9% by weight of active ingredient, more generally from about 10% to about 30% by weight.

As used herein, the term "unit dose" or "unit dosage" refers to physically discrete units that contain a predetermined quantity of active ingredient calculated to produce a desired therapeutic effect. When a unit dose is administered orally or parenterally, it is typically provided in the form of a tablet, capsule, pill, powder packet, topical composition, suppository, wafer, measured units in ampoules or in multidose containers, etc. Alternatively, a unit dose may be administered in the form of a dry or liquid aerosol which may be inhaled or sprayed.

The dosage to be administered may vary depending upon the physical characteristics of the animal, the severity of the animal's symptoms, and the means used to administer the drug. The specific dose for a given animal is usually set by the judgment of the attending physician or veterinarian.

Suitable carriers, diluents and excipients are well known to those skilled in the art and include materials such as carbohydrates, waxes, water soluble and/or swellable polymers, hydrophilic or hydrophobic materials, gelatin, oils, solvents, water, and the like. The particular carrier, diluent or excipient used will depend upon the means and purpose for which the active ingredient is being applied. The formulations may also include wetting agents,

lubricating agents, surfactants, buffers, tonicity agents, bulking agents, stabilizers, emulsifiers, suspending agents, preservatives, sweeteners, perfuming agents, flavoring agents and combinations thereof.

A pharmaceutical composition may be administered using a variety of methods. Suitable methods include topical (e.g., ointments or sprays), oral, injection and inhalation. The particular treatment method used will depend upon the type of infection being addressed.

In parenteral iv applications, the formulations are typically diluted or reconstituted (if freeze-dried) and further diluted if necessary, prior to administration. An example of reconstitution instructions for the freeze-dried product are to add ten ml of water for injection (WFI) to the vial and gently agitate to dissolve. Typical reconstitution times are less than one minute. The resulting solution is then further diluted in an infusion solution such as dextrose 5% in water (D5W), prior to administration.

Pseudomycin compounds have been shown to exhibit antifungal activity such as growth inhibition of various infectious fungi including Candida spp. (i.e., *C. albicans, C. parapsilosis, C. krusei, C. glabrata, C. tropicalis,* or *C. lusitania*); Torulopus spp.(i.e., *T. glabrata*); Aspergillus spp. (i.e., *A. fumigatus*); Histoplasma spp.

(i.e., *H. capsulatum*); Cryptococcus spp. (i.e., *C. neoformans*); Blastomyces spp. (i.e., *B. dermatitidis*); Fusarium spp.; Trichophyton spp., *Pseudallescheria boydii, Coccidioides immits, Sporothrix schenckii,* etc.

Consequently, the compounds and formulations of the present invention may be useful in the preparation of medicaments for use in combating either systemic fungal infections or fungal skin infections. Accordingly, a method is provided for inhibiting fungal activity comprising contacting Compound I of the present invention with a fungus. A preferred method includes inhibiting *Candida albicans, Cryptococcus neoformans,* or *Aspergillus fumigatus* activity. The term "contacting" includes a union or junction, or apparent touching or mutual tangency of a compound of the invention with a fungus. The term does not imply any further limitations to the process, such as by mechanism of inhibition. The methods are defined to encompass the inhibition of fungal activity by the action of the compounds and their inherent antifungal properties.

A method for treating a fungal infection which comprises administering an effective amount of a pharmaceutical formulation of the present invention to a host in need of such treatment is also provided. A preferred method includes treating a *Candida albicans, Cryptococcus neoformans,* or *Aspergillus fumigatus* infection.

The term "effective amount" refers to an amount of active compound which is capable of inhibiting fungal activity. The dose administered will vary depending on such factors as the nature and severity of the infection, the age and general health of the host, the tolerance of the host to the antifungal agent and the species of the host. The particular dose regimen likewise may vary according to these factors. The medicament may be given in a single daily dose or in multiple doses during the day. The regimen may last from about 2-3 days to about 2-3 weeks or longer. A typical daily dose (administered in single or divided doses) contains a dosage level between about 0.01 mg/kg to 100 mg/kg of body weight of an active compound. Preferred daily doses are generally between about 0.1 mg/kg to 60 mg/kg and more preferably between about 2.5 mg/kg to 40 mg/kg. The host may be any animal including humans, companion animals (e.g., dogs, cats and horses), food-source animals (e.g., cows, pigs, sheep and poultry), zoo animals, marine animals, birds and the like.

## EXAMPLES

Unless indicated otherwise, all chemicals can be acquired from Aldrich Chemical (Milwaukee, WI).

### ***Biological Samples***

*P. syringae* MSU 16H is publicly available from the American Type Culture Collection, Parklawn Drive, Rockville,

MD, USA as Accession No. ATCC 67028. *P. syringae* strains 25-B1, 7H9-1, and 67 H1 were deposited with the American Type Culture Collection on March 23, 2000 and were assigned the following Accession Nos.:

| | |
|---|---|
| 25-B1 | Accession No. PTA-1622 |
| 7H9-1 | Accession No. PTA-1623 |
| 67 H1 | Accession No. PTA-1621 |

***Chemical Abbreviations***

The following abbreviations are used through out the examples to represent the respective listed materials:

ACN - acetonitrile

TFA - trifluoroacetic acid

DMF - dimethylformamide

DEAD - Diethylazodicarboxylate

EDCI - 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride

BOC = t-butoxycarbonyl, $(CH_3)_3C-O-C(O)-$

CBZ = benzyloxycarbonyl, $C_6H_5CH_2-O-C(O)-$

FMOC = fluorenylmethyloxycarbonyl

***HPLC Conditions***

Unless indicated otherwise, analytical reverse-phase HPLC work was done using the Waters 600E systems equipped with Waters μBondapak (C18, 3.9 X 300 mm) column. The eluent used was 65:35 acetonitrile/0.1% aqueous TFA solvent

system to 100% acetonitrile over 20 minutes with a flow rate of 1.5 ml/minute and using UV detection at 230 nm.

Preparative HPLC work was performed with a Waters Prep 2000 system using Dynamax 60 angstrom C18 column and identical solvent systems as used in the analytical HPLC system but with a flow rate of 40 ml/min.

### ***Biological Analysis***

*Detection and Quantification of Antifungal Activity:*

Antifungal activity was determined *in vitro* by obtaining the minimum inhibitory concentration (MIC) of the compound using a standard agar dilution test or a disc-diffusion test. A typical fungus employed in testing antifungal activity is *Candida albicans*. Antifungal activity is considered significant when the test sample (50 µl) causes 10-12 mm diameter zones of inhibition on *C. albicans* x657 seeded agar plates.

*Tail Vein Toxicity:*

Mice were treated intravenously (IV) through the lateral tail vein with 0.1 ml of testing compound (20 mg/kg) at 0, 24, 48 and 72 hours. Two mice were included in each group. Compounds were formulated in 5.0% dextrose and sterile water for injection. The mice were monitored for 7 days following the first treatment and observed closely for

signs of irritation including erythema, swelling, discoloration, necrosis, tail loss and any other signs of adverse effects indicating toxicity.

The mice used in the study were outbred, male ICR mice having an average weight between 18-20 g (available from Harlan Sprangue Dawley, Indianapolis, IN).

### *General Procedures*

*General procedures used to protect the pendant amino groups at positions 2, 4 and 5 of Pseudomycin A, A′, B, B′, C or C′*.

Dissolve/suspend pseudomycin compound ($R^1$=H) in DMF (20 mg/ml, Aldrich Sure Seal). While stirring at room temperature add *N*-(Benzyloxycarbonyloxy)succinimide (6 eq). Allow to stir at room temperature for 32 hours. Monitor reaction by HPLC (4.6x50 mm, 3.5 µm, 300-SB, C8, Zorbax column). Concentrate reaction to 10 ml on high vacuum rotovap at room temperature. Put material in freezer until ready to prep by chromatography. Reverse phase preparative HPLC yields an amorphous, white solid after lyophilization.

*General procedures used for chemically deacylating the N-acyl group of the L-serine unit*.

Dissolve/suspend protected Pseudomycin A in water/acetonitrile (2:1 $H_2O$:ACN, about 3.5 mg/ml) and add

TFA (8% by volume) slowly at room temperature. Allow the reaction to stir at room temperature until starting material is consumed. Remove acetonitrile under vacuum at room temperature and lyophilize the material. Dissolve resulting solid in a small amount of DMF (add water and then an equal amount of ACN if necessary). After preparative HPLC and lyophilization, a white, amorphous solid (TFA salt, presumably) is generally observed.

*Solid phase acylation of the Pseudomycin nucleus using HOBt-resin*. The following example uses myristoyl acid; however, the same general procedure may be used with other organic acids.

In a 100 ml double-ended glass fritted reaction tube, myristoyl acid (1.03 g, 3.62 mmol) was dissolved in 50 ml 1:1 DMF/THF. To this solution was added resin HOBt (1.94g, 2.9 mmol), and EDCI (0.556 g, 2.9 mmol) and was shaken overnight. The solvent was drained and the resin was washed with 2xDMF, 2xTHF, and 2x with 1:1 DMF/THF. CBZ-protected Pseudomycin nucleus (1.0 grams, 0.723 mmol) was dissolved in 50 ml DMF/THF (20 mg/ml), and added to the resin bound activated ester and mixed overnight on a rotator or shaker. The product was drained away from the resin and the remaining resin was washed with 2xDMF, 2xTHF, and 2x1:1 DMF/THF. The combined filtrates were isolated by reverse

phase HPLC and lyophilized to yield (129 mg, 10%) Myristoyl acylated CBZ-protected Pseudomycin product.

*Acylation of the Pseudomycin nucleus using an activated ester (HOBt-mesylate)*. The following example uses glycine myristoyl acid; however, the general procedures may be used with other organic acids.

In a 500 ml round bottom flask, glycine myristoyl acid (0.309 g, 1.1 mmol) was dissolved in 100 ml of DMF. To this solution was added HOBt-mesylate (0.229 g, 1.1 mmol )and triethylamine (0.081g, 0.8mmol )The solution was stirred rapidly overnight under 1 atm $N_2$. DMF and TEA were dried off using the high vacuum. The residual oil was azeotroped 3x with toluene till a white solid formed. To the solid was added 100 ml of DMF and 1g of CBZ-protected Pseudomycin nucleus. The solution was stirred overnight, and dried on the high vacuum. The product was isolated by reverse-phase HPLC and lyophilized to yield (233 mg, 20%) Myristoyl acylated CBZ-protected Pseudomycin product.

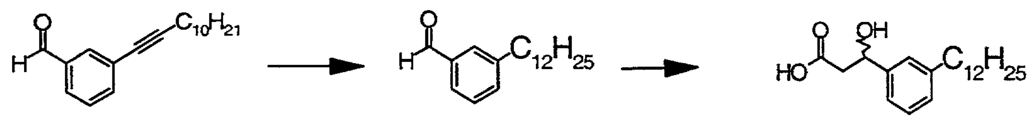
*General procedures used to deprotect the pendant amino groups at position 2, 4 and 5 by hydrogenation*.

Dissolve CBZ-protected acylated-derivative in a cold 13% acetic/methanol solution (5 mg/ml) and add an equivalent amount of 10% Pd/C. Charge the reaction with hydrogen by

degassing reaction and replacing volume with $H_2$ ,4-7 times. Allow reaction to proceed at room temperature. Monitor the reaction by HPLC and mass spectrometry every 15 minutes until the starting material is consumed. When the reaction is complete, remove balloon and filter reaction with 0.45 µm filter disk (Acrodisk GHP, GF by Gelman). Concentrate to about 1/10$^{th}$ volume and prep by HPLC. Lyophilize fractions containing product.

**Preparations**

*Preparation of Side-Chain Precursor (1c):*



**1a** **1b** **1c**

A 250 ml round bottom flask containing 100 ml of degassed ACN was charged with *m*-bromobenzaldehyde (5.000 g, 27.02 mmol), triethylamine (5.490 g, 54.25 mmol) and 1-dodecyne (5.000g, 30.06 mmol). To this mixture was added $PdCl_2$ (243.1 mg, 1.370 mmol), triphenylphosphine (718.8 mg, 2.740 mmol) and CuI (173.8 mg, 0.9120 mmol). The reaction was then heated to reflux and allowed to react overnight. The reaction was then cooled to room temperature and the solvent was removed *in vacuo*. The resulting residue was taken up in methylene chloride and washed 2 X 1N HCl and 1 X

brine. The organic layer was dried over $MgSO_4$. The drying agent was then filtered off and the solvent was removed *in vacuo*. Purification on a silica gel column eluting with 3% EtOAc/hexanes yielded 3.73 grams of a the titled compound as a brown oil. The spectral data was consistent with the structure for *m*-(1-Dodecynyl)benzaldehyde (1a).

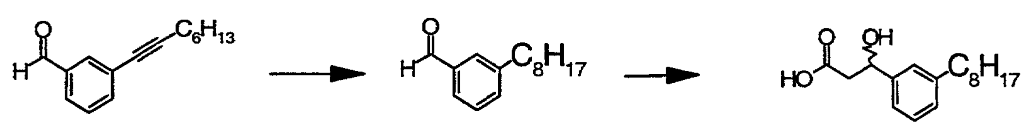
To 100 mL of EtOAc was added the above compound (1.00 g, 3.70 mmol) and 0.1 g of 5% $Pd/Al_2O_3$. The reaction mixture was subjected to 50psi of $H_2$ at room temperature for 1 hour. The reaction mixture was filtered over celite to remove the catalyst and the celite was rinsed with copious amounts of EtOAc. Removal of the EtOAc via a rotary evaporator yielded 882.1 mg of the product. This was used in the next step without further purification. The spectral data was consistent with *m*-Dodecylbenzaldehyde (1b).

An oven dried 50 ml round bottom flask was charged with 6.0 ml anhydrous THF under a nitrogen atmosphere at -78 $^0$C and lithium diisopropyl amine (1.2 mL of a 2M solution in heptane/THF/ethylbenzene, 2.41 mmol) was then added. To this was added *t*-butyl acetate (0.331 ml, 2.45 mmol) and the resulting solution was raised to approx. -40$^0$C and maintained at this temperature for 1 hour. The above compound (501.9mg, 1.83 mmol), dissolved in 4 mL anhydrous THF and precooled to -40$^0$C, was then added dropwise to the anion. The reaction mixture was allowed to stir for 1 hour

before quenching with 2 ml of saturated aqueous ammonium chloride and 10 ml water. The reaction mixture was partitioned between ether and water. The organic layer was washed 1 X brine and dried over sodium sulfate. The drying agent was then filtered off and the solvent was removed *in vacuo*. Purification on a silica gel column eluting with 5% EtOAc/hexanes yielded 238.1 mg of a yellow oil. The spectral data was consistent with *t*-Butyl 3-hydroxy-3-(*m*-dodecylbenzyl) propionoate.

A solution of precooled (0ºC) of 4 ml TFA was added to a 50 ml round bottom flask containing crude *t*-Butyl 3-hydroxy-3-(*m*-dodecylbenzyl) propionoate. The reaction was allowed to stir at this temperature for 25 min at which time TLC (10% EtOAC/hexanes) indicated the consumption of the starting ester. The TFA was removed *in vacuo* yielding an oil (**1c**).

*Preparation of Side-Chain Precursor (2c):*

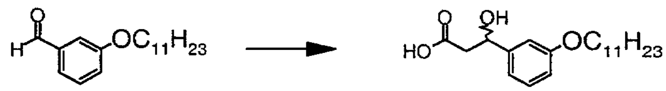



**2a** **2b** **2c**

Compound **2a** is synthesized using the same procedures as described above for Compound **1a** using 1-octyne instead of 1-

dodecyne. Compounds 2b and 2c are synthesized using the same procedures described above for 1b and 1c, respectively.

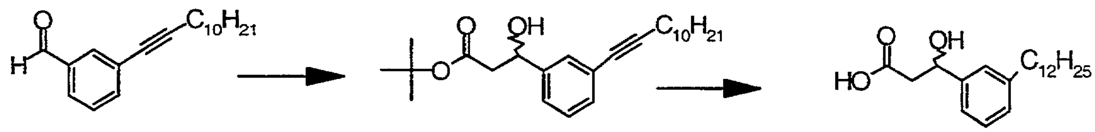
*Preparation of Side-Chain Precursor (3b)*:



**3a** **3b**

A 500 mL round bottom flask containing 100 mL of acetone was charged with *m*-hydroxybenzaldehyde (5.00g, 40.94 mmol), 1-bromoundecane (9.65g, 41.02 mmol) and $K_2CO_3$ (8.48g, 61.36 mmol). The reaction mixture was heated to reflux and allowed to react for 10 h. The reaction was the cooled and the acetone was removed *in vacuo*. The resulting residue was partitioned between ether/water. The organic layer was washed 2 X saturated aqueous $NaHCO_3$ and 1 X Brine. The organic layer was dried over $MgSO_4$. The drying agent was then filtered off and the solvent was removed *in vacuo*. Purification on a silica gel column eluting with 3% EtOAc/hexanes yielded 1.6876 of a yellow oil. The spectral data was consistent with Compound **3a**. This aldehyde was then carried through using the same procedures described in the preparation of **1c** to produce Compound **3b**.

*Preparation of Side-Chain Precursor (4c):*

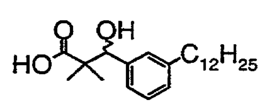


**1a** **4a** **4b**

Compound **4a** is synthesized using the same procedures as described above for Compound **1c** with the exception that Compound **1a** is not hydrogenated prior to condensation with *t*-butyl acetate.

A 50 ml round bottom flask was charged with *t*-Butyl 3-hydroxy-3-(*m*-dodecynylbenzyl) propionoate **4a**(346.8 mg, 0.897) and dissolved in 10 ml MeOH /1 mL glacial AcOH. Standard hydrogenolysis with 10% Pd/C (304.5 mg) for 24 h. Removal of the catalyst via filtration and removal of the solvent *in vacuo* led to 302.4 mg of *t*-Butyl 3-(*m*-dodecylbenzyl) propionoate. The t-butyl ester was then removed by treatment with TFA to produce Compound **4b**.

*Preparation of Side-Chain Precursor (5a):*

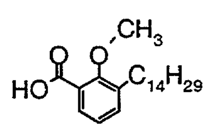


**5a**

To a 50 ml round bottom flask containing 5 mL THF at -78$^0$C was added *sec*-BuLi (1.56 mmol, 1.2 mL of a 1.3 M sol'n in cyclohexane). To this mixture was added *t*-butyl

bromoisobutyrate (in 2 ml THF at -78$^0$C ) dropwise. This reaction mixture was allowed to stir for 30 min at which time Compound **1b** (318.9 mg, 1.16 mmol in 2 ml THF at -78$^0$C ) was then added to the reaction mixture dropwise. The resulting reaction mixture was allowed to stir at -78$^0$C for 30 min and then raised to 0$^0$C over a period of 30 min and allowed to stir at that temperature for 1.75 h. The reaction was then quenched with 2 ml of saturated aqueous ammonium chloride and allowed to warm to room temperature. The reaction mixture was the partitioned between ether/water and the organics were washed 1x brine and dried over $MgSO_4$. The drying agent was then filtered off and the solvent was removed *in vacuo* to yield 370.5 mg of crude material as a racemic mixture. The t-butyl ester was then removed by treatment with TFA to produce Compound **5a**.

*Preparation of Side-Chain Precursor (6a):*

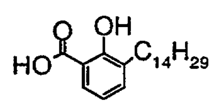


**6a**

To a 250 mL round bottom flask containing 100 mL MeOH and 1 mL of conc. $H_2SO_4$ was added *m*-bromoanisic acid (5.00 g, 21.6 mmol). The resulting mixture was heated to reflux and allowed to stir for 24 h. The reaction was cooled and the

solvent was removed *in vacuo*. The resulting solid was taken up in ether and the organics were washed 2x water, 1x saturated aqueous $NaHCO_3$ and 1x brine and dried over $MgSO_4$. The drying agent was then filtered off and the solvent was removed *in vacuo* to yield 4.72g of crude material which was used without further purification. This was then coupled 1-tetradecyne and underwent hygrogenolysis to the alkene analogously to previous examples. The methyl ester was converted to the carboxylic acid by suspending the methyl ester (538.4 mg, 1.48 mmol) in 20 ml of a 30% HBr in AcOH solution and heating to reflux. After 24 h at reflux the solution was poured into 150 ml of water and extracted out with 2x 200 ml $CH_2Cl_2$. The organics were washed with copious amounts of water and dried over $MgSO_4$. The drying agent was then filtered off and the solvent was removed *in vacuo* to yield 398.7g of crude material **6a** which was used without further purification.

*Preparation of Side-Chain Precursor (7a):*

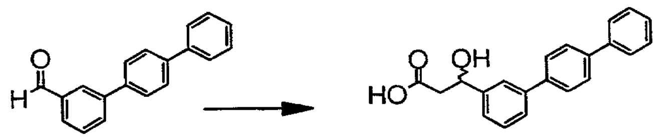



**7a**

Compound **6a** (385.2 mg) was added to pyridinium hydrochloride solid. The 2 solids were melted by heating to

~ 220 $^{0}$C and allowing the mixture to react for 3 h. The reaction was then cooled and partitioned between $CH_2Cl_2$/1N HCl. The organics were then washed 5x 1N HCl and dried over $MgSO_4$. The drying agent was then filtered off and the solvent was removed *in vacuo* to yield 158.8 mg of crude material(**7a**) which was used without further purification.

*Preparation of Side-Chain Precursor (8b):*

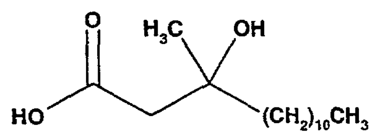


**8a** **8b**

A 250 ml round bottom flask was charged with biphenyl boronic acid (2.00 g, 10.1 mmol) and $Pd(PPh)_4$ (980.0 mg, 0.848 mmol) in 60 mL toluene/30 mL 2 M aqueous $Na_2CO_3$. To this slurry was added *m*-bromobenzaldehyde (in 10 mL MeOH). The reaction was heated to reflux and allowed to react for 20 h. The reaction was cooled and the organic layer was washed 2x water, 1x brine and dried over $MgSO_4$. The drying agent was then filtered off and the solvent was removed *in vacuo*. The resulting solids were rinsed with cold hexanes to remove any residual *m*-bromobenzaldehyde. The solid was then slurried in hot hexanes and filtered hot to remove any solids. The filtrate was then removed *in vacuo* to yield the desired aldehyde **8a**. Compound **8a** was converted to **8b** using

the same procedures as described in the preparation of **1c** to yield a mixture of inseparable diastereomers.

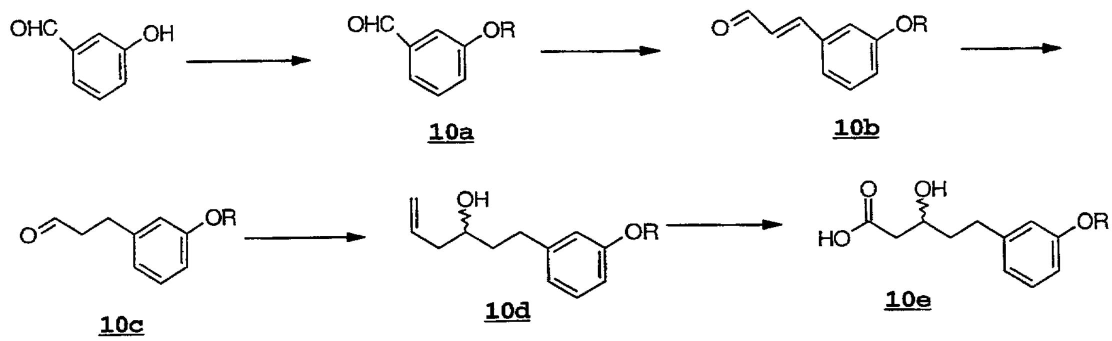
*Preparation of Side-Chain Precursor (9a):*



**9a**

A solution of t-butyl acetate (2.02 ml, 0.015 mol) in anhydrous THF (25 ml) was cooled to -78 $^0$C and n-butyl lithium (1.6 M in hexane)(9.35 ml, 0.015 mol) was added dropwise. After 45 min a THF solution of 2-tridecanone (2.0 g, 0.01 mol) was added dropwise. The stirring was continued at low temperature for 1 hr and then the reaction was allowed to warm to room temperature over 15 min. Excess 1N HCl was added to quench the reaction and the aqueous solution was extracted with ether (2x). The ether extracts were dried over $MgSO_4$ and reduced *in vacuo* to give a crude oil. Purification by column chromatography over silica (5% ethyl acetate/hexane) gave 1.01 g (32% yield) of the t-butyl ester. NMR was consistent with the desired product. The *t*-butyl ester was then treated with trifluoroacetic acid to cleave the *t*-butyl ester to produce Compound **9a** with a quantitative yield.

*Preparation of Side-Chain Precursor (10e):*



R = n-$C_5H_{11}$

To a THF solution (16 mL) of m-hydroxybenzaldehyde (1.00 g, 8.20 mmol) was added at rt DEAD (1.29 mL, 8.20 mmol), $PPh_3$ (2.15 g, 8.20 mmol) and n-pentanol (723 mg, 8.20 mmol). The reaction was stirred overnight at rt. After silica gel purification, 1.02 g (65%) of the desired product **10a** was obtained.

To a THF solution of **10a** (6.70 g, 34.90 mmol) was added at 0°C $Ph_3P$=CHO (10.6 g, 34.90 mmol). After stirring at rt overnight, the reaction mixture was filtered and conc. *in vacuo* to give a residue, which was purified by silica gel chromatography to give 3.57 g (47%) of the desired unsaturated aldehyde **10b**.

An EtOAc solution (30 mL) of **10b** (3.37 g, 15.5 mmol) was subjected to hydrogenation (1.5 atm) using 10% Pd/C (1.64 g, 1.55 mmol). The reaction was stirred overnight. At this point, the reaction mixture was filtered through a pad of Celite. The filtrates and rinses were conc. in vacuo. The

residue thus obtained was purified by silica gel chromatography (10% EtOAc/Hexanes) to afford 2.37 g (70%) of **10c**.

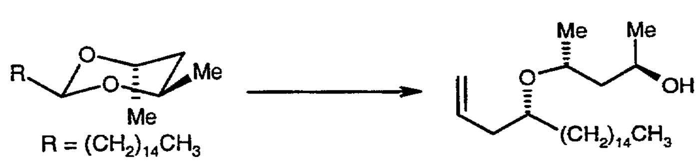
To a dichloromethane solution (23 mL) of aldehyde **10c** (1.26 g, 5.71 mmol) was added at 0°C allyltrimethylsilane (0.91 mL, 5.71 mmol), followed by $TiCl_4$ (0.63 mL, 5.71 mmol). After stirring for 1 hr at 0°C, the reaction was quenched with saturated $NaHCO_3$ solution. The mixture was diluted with dichloromethane (75 mL). The organic layer was washed sequentially with saturated $NaHCO_3$, water and brine. The organic layer thus obtained was dried, conc. and purified via silica gel chromatography (10% EtOAc/Hexanes) to afford 0.91 g (61%) of the desired allylic alcohol **10d**.

Allylic alcohol **10d** (0.91 g, 3.47 mmol) was dissolved in aqueous acetone (7 mL each). To this solution was added NMO (704 mg, 5.21 mmol) and a THF solution of $OsO_4$ (44 mg, 0.17 mmol). Atfer stirring at rt for 2 hr, the reaction was quenched with $NaHSO_3$ (750 mg) to quench the excess oxidant. The reaction mixture was diluted with brine (10 mL) and extracted with EtOAc (3 x 50 mL). The combined organic layers were dried and conc. in vacuo to provide 850 mg (82%) of the desired triol intermediate. The triol (850 mg, 2.87 mmol) thus obtained was dissolved in MeOH (30 mL) and water (6 mL). This solution was treated with $NaIO_4$ (1.38 g, 6.46

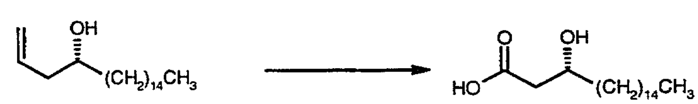
mmol) at rt. After 1 hr, the reaction mixture was filtered through a pad of Celite. The filtrates were carefully conc. in vacuo to yield the corresponding beta-hydroxy aldehyde. This material was dissolved in a mixture of t-BuOH (14 mL) and cyclohexene (2 mL). To the above solution was added an aqueous solution (15 mL $H_2O$) containing $KH_2PO_4$ (2.33 g, 17.7 mmol) and $NaClO_2$ (2.08 g, 23.0 mmol). The reaction was stirred at rt for 5 hr and then acidified to pH =3 with 1N HCl. The reaction mixture was extracted with EtOAc (3 x 50 mL). The combined extracts were washed with water and brine. The organic layer was dried and conc. *in vacuo* to give the crude acid **10e** (1.5 g, ~3.4 mmol).

Compounds where R is n-$C_6H_{13}$, n-$C_7H_{15}$, n-$C_8H_{17}$, n-$C_9H_{19}$, n-$C_{10}H_{21}$, and n-$C_{14}H_{29}$ were also made using the same procedures as described above.

*Preparation of Side-Chain Precursor (11d):*




**11a** **11b**




**11c** **11d**

To a dichloromethane solution (190 mL) of the chiral acetal **11a** (6.22 g, 19.1 mmol) was added at -78°C trimethylallylsilane (10.9 mL, 68.69 mmol), followed by neat $TiCl_4$ (2.94 mL, 26.71 mmol). The reaction was stirred at -78°C for 1 hr and then at -40°C for 2 hr. At this point, the reaction was quenched with methanol (15 mL) and diluted with dichloromethane (200 mL). The resulting reaction mixture was washed with 1N HCl (2 x 50 mL), water and brine. The organic layer was dried and conc. in vacuo to give a residue, which was purified by silica gel chromatography (10% EtOAc/Hexanes) to give 5.51 g (78%) of the desired product **11b**.

$^1$H NMR of **11a** ($CDCl_3$): δ 4.73 (m, 1H), 4.21 (m, 1H), 3.86 (m, 1H), 1.75 (m, 1H), 1.60-1.10 (m, 35H), 0.80 (m, 3H). $^1$H NMR of **11b** ($CDCl_3$): δ 5.81 (m, 1H), 5.05 (m, 2H), 4.12 (m, 1H), 3.86 (m, 1H), 3.41 (m, 1H), 2.22 (m, 2H), 1.67-1.18 (m, 36H), 0.88 (m, 3H).

To a dichloromethane solution (155 mL) of **11b** (8.56 g, 23.3 mmol) was added PCC (10.0 g, 46.5 mmol). The reaction was stirred at rt for 18 hr, and then filtered through a pad of Celite. The filtrates were concentrated in vacuo to give a reddish residue, which was purified by silica gel chromatography (10% EtOAc/Hexanes) to give 8.36 g (80%) of the methyl ketone intermediate (structure not shown). The

intermediate obtained herein (8.36 g, 22.8 mmol) was dissolved in THF (60 mL) and MeOH (30 mL). To this solution was added 7.5 M KOH (15 mL). After stirring 3 hr at rt, the solvent was partially removed. The remaining reaction mixtures were diluted with EtOAc/Et2O (3:1 ratio, 350 mL). The organic layer was washed with water (3 x 50 mL) and brine. The resulting organic layer was dried and conc. in vacuo to give a residue, which was purified by silica gel chromatography (10% EtOAc/Hexanes) to afford 6.22 g (96%) of the desired product **11c** as white solids.

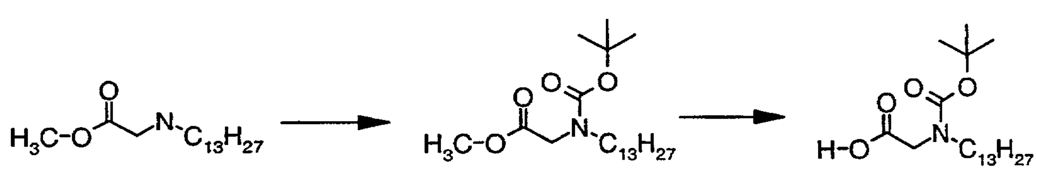
$^1$H NMR of **11c** ($CDCl_3$): $\delta$ 5.75 (m, 1H), 5.06 (m, 2H), 3.56 (m, 1H), 2.23 (m, 1H), 2.07 (m, 1H), 1.75-1.17 (m, 28H), 0.80 (m, 3H).

Carbinol **11c** (6.22 g, 22.0 mmol) was dissolved in an aqueous THF solution (5.5 mL water and 55 mL THF). To this solution was added NMO (4.42 g, 33.0 mmol), followed by $OsO_4$ (280 mg dissolved in THF, 1.10 mmol). The reaction stirred at rt overnight. At this time, sodium bisulfide (4 g) was added. The reaction was stirred for 2 hr, and then diluted with EtOAc (300 mL). The whole mixture was washed with water (2 x 40 mL) and brine. The resulting organic layer was dried and conc. in vacuo to give the corresponding triol intermediate. This material was dissolved in MeOH (200 mL) and water (40 mL). To this solution was added $NaIO_4$ (10.6 g,

49.5 mmol). After stirring at rt for 1hr, the reaction was filtered through Celite and purified by short column silica gel chromatography (30% EtOAc/Hexanes) to afford ~10 g (>100%) crude beta-hydroxyl aldehyde. The impuried aldehyde thus obtain was dissolved in t-BuOH (100 mL) and cyclohexene (14 mL). To this solution at rt was added an aqueous solution (50 mL) of $NaClO_2$ (15.97 g, 176 mmol) and $KH_2PO_4$ (17.8 g, 132 mmol). The reaction was stirred at rt for 6 hr and then quenched at 0°C with 5N HCl to pH=4. The reaction was extracted with 3:1 mix-solvent EtOAc/$Et_2O$ (3 x 250 mL). The organic layer was washed with brine and dried and conc. to provide 7.3 g (>100%) of the crude acid **11d**, which was used directly for the coupling reaction.

$^1H$ NMR of **11d** ($CDCl_3$): δ 3.95 (m, 1H), 2.60-2.35 (m, 2H), 1.40-1.10 (m, 28H), 0.82 (m, 3H).

*Preparation of Side-Chain Precursor (12c):*




**12a** **12b** **12c**

A 1 liter round bottom flask was charged with tridecanal (5.00 g, 25.2 mmol) and the HCl salt of Gly-Ome (12.66 g, 100.8 mmol) in 600 ml anhydrous MeOH. To this

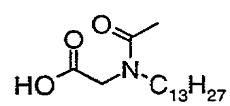
reaction mixture was added $NaCNBH_3$ (1.787 g, 28.44 mmol) and the reaction was allowed to stir overnight at room temperature. The solids were filtered off and the solvent was removed *in vacuo*. The resulting residue was partitioned between $CH_2Cl_2$/saturated aqueous $NaHCO_3$. The organic layer was washed 2x $NaHCO_3$ and dried over $MgSO_4$. The drying agent was then filtered off and the solvent was removed *in vacuo*. Purification on a silica gel column eluting with 50% EtOAc/hexanes yielded 2.94 g of a white solid (**12a**).

A 50 ml round bottom flask was charged with the glycine derivative **12a** (504.6 mg, 1.86 mmol), triethylamine (224.6 mg, 2.22 mmol) in 10 ml anhydrous THF. To this was added $(BOC)_2O$ (494.3 mg, 2.26 mmol) in one portion. The reaction was allowed to stir for 18 h at which time the solvent was removed *in vacuo*. The resulting oil was taken up in EtOAc and washed 2x 1N HCl, 1x water, 1x brine and dried over $MgSO_4$. The drying agent was then filtered off and the solvent was removed *in vacuo* to yield 463.3 mg of a colorless oil (**12b**) which was used without purificaton.

A 50 ml round bottom flask containing 5 ml THF was charged with the methyl ester **12b** (463.3 mg, 1.25 mmol). To this was added 1.8 ml of a 1N LiOH solution. The resulting reaction mixture was allowed to stir overnight. The reaction was quenched by the addition of 1.8 ml of a 1N HCl

solution. The THF was then removed *in vacuo* and the resulting aqueous layer was extracted 2x $CH_2Cl_2$. The organics were then dried over $MgSO_4$. The drying agent was then filtered off and the solvent was removed *in vacuo* to yield 246.2 mg of a colorless oil (**12c**) which was used without purification.

*Preparation of Side-Chain Precursor (13a):*



**13a**

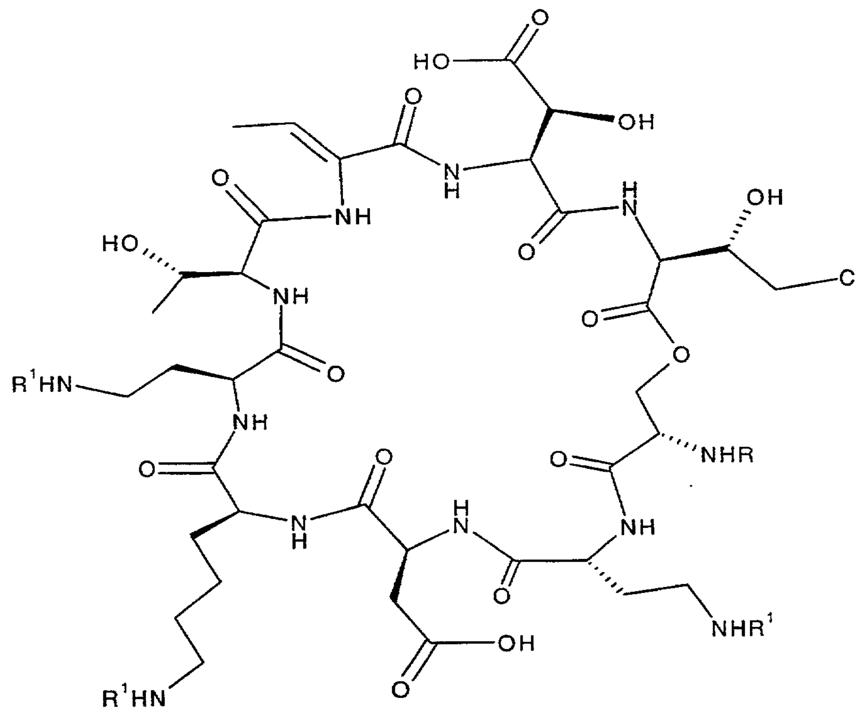
The methyl ester **12b** from above (499.7 mg/1.84 mmol) was dissolved in a 1:1 mixture of acetic anhydride/pyridine in a 50 mL round bottom flask. The reaction was allowed to stir overnight at which time the solvent was removed *in vacuo*. The resulting oil was taken up in $CH_2Cl_2$ and washed 2x 1N HCl, 1x water, 1x brine and dried over $MgSO_4$. The drying agent was then filtered off and the solvent was removed *in vacuo* to yield 319.9 mg of a colorless oil (**13a**) which was used without purificaton.

*General Preparation for Glycine side-chain precursors (14-a):*

For example the following procedure describes the synthesis of tridecanoyl-glycine-acid from Fmoc-glycine-wang resin and tridecanoic acid.

In a 100mL double-ended glass fritted reaction tube, Fmoc-glycine-wang resin (10g, 4.4 mmol) was added to 50 mL of 30% Piperdine/DMF. The reaction was shaken for 20 minutes, and was washed 3x with DMF, 3x with isopropanol, and 3x with DMF. To the resin was added a solution of tridecanoic acid (4.708g, 22 mmol) in 50 mL DMF. To this mixture was added HOBt (2.97 g, 22 mmol) and DIC (2.77 g, 22mmol). The reaction vessel was put on a shaker overnight. The resin was then washed 3x with DMF, 3x with dichloromethane, 3x with MeOH, 3x with THF, and 3x with dichloromethane. The resin beads were dried in a vacuum oven for 1 hour. To the resin was added 100 mL of 95% TFA/H2O. The reaction was shaken for 1.5 hours, and the non-resin product was washed with TFA and collected. The product was dried in a vacuum oven to a white residue, and azeotroped with Toluene to yield tridecanoyl-glycine-acid (1.14 g, 95%) $^{1}$HNMR (THF) 0.82-0.92 (t, J= 7.2, 3H), 1.2-1.4 (s, 18H), 1.52-1.62 (m, 2H), 2.10-2.17 (t, J=7.2 , 2H), 3.83-3.89 (d, J=7.1 , 2H), 7.04-7.17 (s, 1H), 10.8-10.9 (s, 1H).

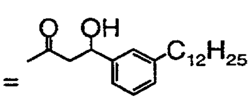
The following structure II will be used to describe the products observed in Examples 1 through 17.




**II**

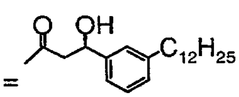
***Example 1***

*Synthesis of diastereomers 1-1 and 1-2:*

R = 


$R^1$ = CBZ

**1-1**

R = 

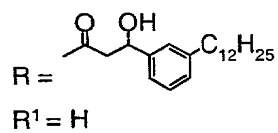

$R^1$ = CBZ

**1-2**

Hydroxybenzotriazole (55.8 mg, 0.413 mmol) and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (82.4 mg, 0.430 mmol) was added to Compound **1c** in 4 ml anhydrous DMF. This reaction mixture was allowed to stir for 10 hours at room temperature at which time Z-PSN (375.2 mg, 0.271 mmol) was then added. HPLC indicated the

consumption of CBZ-protected pseudomycin nucleus (Z-PSN) after a period of 5 hours. The solvent was removed *in vacuo* and the resulting residue was taken up in 1:1 ACN/$H_2O$ and purified via preparatory HPLC. This yielded 2 major peaks whose mass spectral data suggests that these peaks correspond to the 2 diasteromers **1-1** (88.3 mg) and **1-2** (166.3mg).

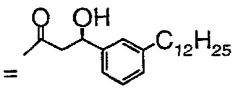
*Deprotection of Diasteromer 1-1:*




**1-3**

A 50 ml round bottom flask was charged with 10 ml MeOH /1 ml glacial AcOH and diastereomer **1-1** (82.0 mg, 0.048 mmol). After degassing 89.1 mg of 10% Pd/C was added to the reaction mixture and subjected to 1 atm $H_2$ for 30 minutes. Removal of the catalyst via filtration and purification via preparatory HPLC and subsequent lyophilization provided 21.7 mg of Compound **1-3** ($R^1$ = H). MS (Ionspray) calcd for $C_{58}H_{94}ClN_{12}O_{19}$ $(M+H)^+$ 1297.64, found 1297.8.

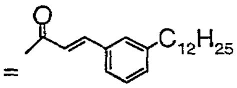
*Deprotection of Diasteromer 1-2:*



R =

$R^1$ = H

**1-4**



R =

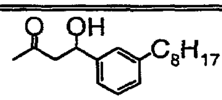
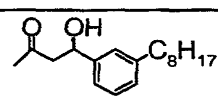
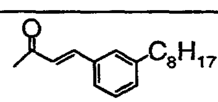
$R^1$ = H

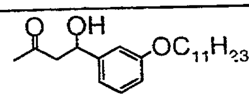
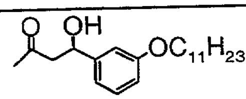
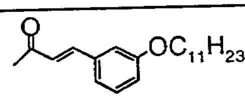
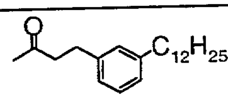
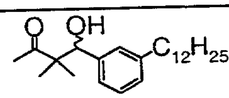
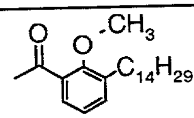
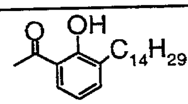
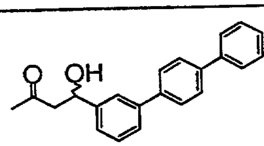
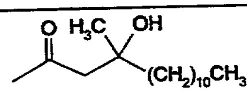
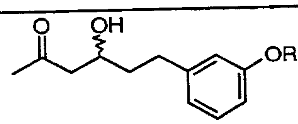
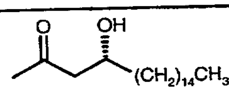
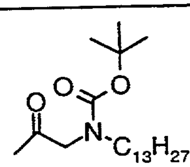
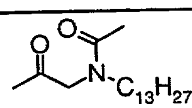
**1-5**

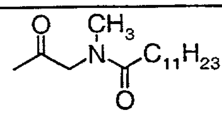
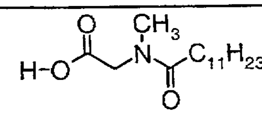
Diastereomer **1-2** (152.8 mg, 0.089mmol) was subjected to hydrogenolyis as described for diasteromer **1-1** using 152.8 mg of 10% Pd/C for 30 min. HPLC indicated consumption of starting material and formation of two product peaks which, after preparatory HPLC and lyophilization, were found to be Compound **1-4** (18.0 mg ) and Compound **1-5** (11.3 mg). Compound **1-4**: MS (Ionspray) calcd for $C_{58}H_{94}ClN_{12}O_{19}$ $(M+H)^+$ 1297.89, found 1297.8. Compound **1-5**: MS (Ionspray) calcd for $C_{58}H_{92}ClN_{12}O_{18}$ $(M+H)^+$ 1279.63, found 1281.7.

Each of the compounds listed in Table 1 below was synthesized using the same acylation and deprotection procedures as described above using the indicated side-chain precursor. For each compound listed in Table 1, $R^1$ is a hydrogen.

**Table 1**

| Example No. | R = | Side-Chain Precursor |
|---|---|---|
| 2-1 |  | 2c |
| 2-2 |  | 2c |
| 2-3 |  | 2c |

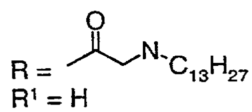
| | | |
|---|---|---|
| 3-1 |  | 3b |
| 3-2 |  | 3b |
| 3-3 |  | 3b |
| 4-1 |  | 4b |
| 5-1 |  | 5a |
| 6-1 |  | 6a |
| 7-1 |  | 7a |
| 8-1 |  | 8b |
| 9-1 |  | 9a |
| 10-1 |  | 10e |
| 11-1 |  | 11d |
| 12-1 |  | 12c |
| 13-1 |  | 13a |

| | | |
|---|---|---|
| 14-1* |  |  (available from Sigma) |

*Although this compound and the compound where the N-methyl group is absent shows little activity, Compounds where the alkyl chain is increased progressively from C11 to C15 show significant increased activity. Side chains of this class may be prepared using the preparation described in preparation 14-a.

***Example 15***

*Synthesis of Compound 15-1:*



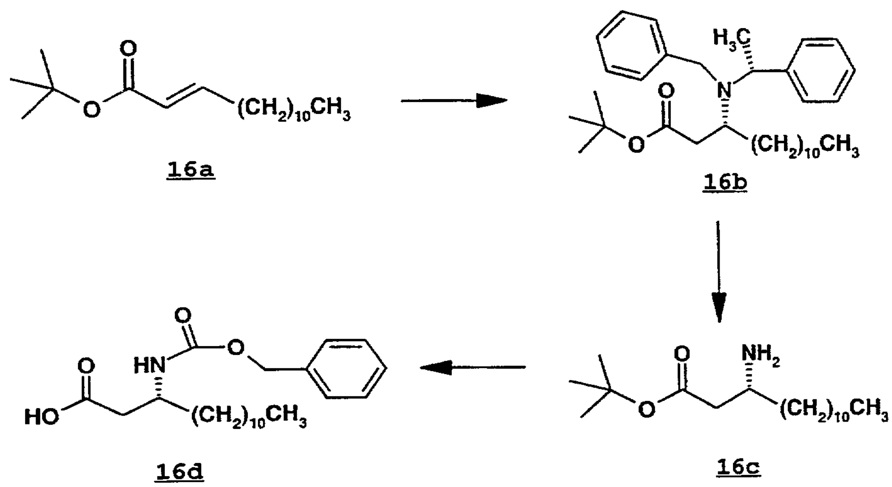
**15-1**

To a 50 ml round bottom flask was added 31.3 mg (0.0237 mmol) of Compound **12-1** and 5 ml TFA (precooled to 0 $^0$C). The reaction mixture was allowed to stir at this temperature for a period of 15 min at which time the TFA was removed *in vacuo*. The residue was then taken up in water and lyophilized to yield 24.3 mg of Compound **15-1**. No further purification was necessary.

***Example 16***

Example 16 illustrates the attachment of a Beta-amino substituted side-chain.

*Preparation of Compounds 16a, 16b, 16c and 16d:*




A solution of t-butoxycarbonylmethylenetriphenyl-phosphorane (5.0 g, 13.3 mmol) and dodecyl aldehyde (2.2 ml, 10 mmol) in toluene (50 ml) was refluxed for 1 hr 20 min. The solution was filtered through a plug of silica to remove the phosphorus reagents and then reduced *in vacuo* to give a crude oil. The oil was purified over a silica column by elution with 2% ethyl acetate in hexane to give 2.36 g (84% yield) of compound **16a**. MS- 283.4 (M+1) NMR consistent with structure.

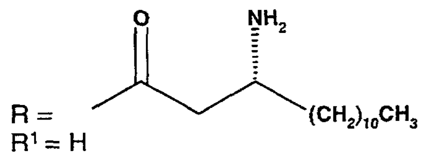
Butyl lithium (1.6 M in hexane)(1.19 ml, 1.9 mmol) was added slowly to a solution of ( R )-benzylmethyl benzylamine (0.42ml, 2.0 mmol) in THF (5 ml) cooled to -78 $^{0}$C. A THF solution of **16a** (500 mg, 1.77 mmol) was then added dropwise. The mixture was stirred at -78 $^{0}$C for 1 hr. The reaction mixture was then poured into sat. $NH_4Cl$ solution and extracted with ether (2x). The ether solution was dried over $MgSO_4$ and reduced *in vacuo* to give 0.95 g of **16b** as a

crude oil which was carried to the next step without purification.

An ethanol (25 ml) solution of **16b** (0.95 g) and $Pd(OH)_2/C$ (0.47 g) was put under 60 psi of $H_2$ at 55 $^0C$ for 18 hr. The suspension was filtered and then reduced in vacuo to give 280 mg (53% yield) of crude **16c** which was carried directly to the next step.

Compound **16c** (280 mg, 0.93 mmol) and N-benzyloxy-carbonyloxysuccinimide (274 mg, 1.1 mmol) were mixed in THF (10 ml) and stirred overnight. The solvent was removed *in vacuo* and the product oil was purified by column chromatography over silica using 5% ethyl acetate in hexane as the eluant to give 268 mg (66% yield) of t-butyl ester of **16d**. NMR was appropriate for the expected structure. The ester (97 mg, 0.224 mmol) was dissolved in trifluoroacetic acid (2 ml) at 0 $^0C$ for 0.5 hr to remove the t-butyl ester (**16d**) with a quantitative yield.

*Synthesis of Compound 16-1:*




Compound **16d** was dissolved in DMF (2 ml). Hydroxybenzotriazole (36.4 mg, 0.269 mmol) and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (47.1 mg, 0.246 mmol) were added and the solution was stirred 18 hr. The CBZ protected pseudomycin nucleus (Z-PSN) (277 mg, 0.185 mmol) was added and the reaction was stirred for an additional 18 hr. The reaction product was purified by HPLC and lyophilization gave 136.3 mg (42% yield) of the acylated CBZ protected pseudomycin derivative as a white solid. MS- 1743 (M) and the NMR was consistent with the proposed structure.

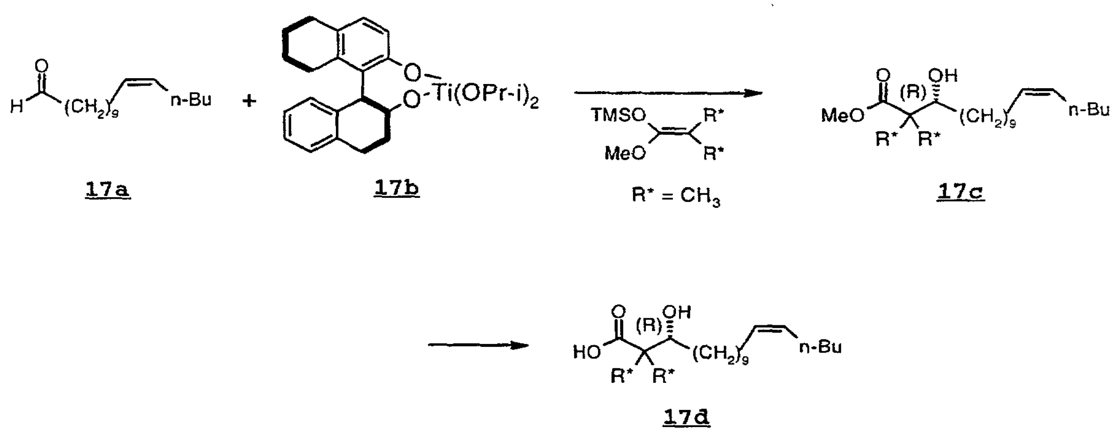
A methanol (10 ml) and acetic acid (1.5 ml) solution of the acylated compound (130 mg, 0.0746 mmol) with 10% Pd/C (120 mg) was put under a balloon of hydrogen for 20 min. The solution was filtered and purified by preparative HPLC. Lyophilization gave 23 mg (18% yield) of the trifluoroacetic acid salt of **16-1** as a white solid. MS- 1206.8 (M) and the NMR was consistent with the proposed structure. Compound **16-1** showed little or no activity against *Candida Albicans*

and *Cryptococcus neoformans* which is a significant reduction in activity as compared to the β-hydroxy analog.

### *Example 17*

Example 17 illustrates the attachment of a chiral side-chain.

<u>*Preparation of side chain precursor 17d:*</u>




To a THF solution of (S)-binaphthol (240 mg, 0.84 mmol) was added 4A molecular sieves (4 g), followed by addition of neat Ti(OPr-I)$_4$ (0.25 mL, 0.84 mmol). The reaction mixture turned red immediately and remained to be red. The chiral catalyst (<u>17b</u>) thus prepared was used for the subsequent reaction.

To a freshly prepared THF solution (4 mL) of (S)-binaphthol-Ti catalyst <u>17b</u> (0.42 mmol) was added at -78°C a THF solution containing trimethylsilyldimethylketene acetal (0.43 mL, 2.1 mmol) and the unsaturated aldehyde <u>**17a**</u> (500 mg, 2.1 mmol) over 15 min. The reaction was stirred at -78°C

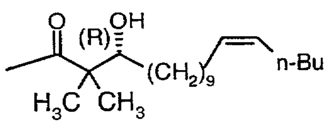
for 1 hr and then at rt overnight. At this point, the reaction was quenched with saturated $NaHCO_3$ solution and extracted with EtOAc (100 mL). The organic layer was washed with $NaHCO_3$, brine, and dried with anhydrous MgSO4. Upon filtration and conc. in vacuo. The residue was purified with silica gel chromatography (10% EtOAc/Hexanes) to give 257 mg (36%) of the desired product **17c**.

$^1$H NMR of **17c** ($CDCl_3$): $\delta$ 5.29 (m, 2H), 3.65 (s, 3H), 3.53 (t, J = 7.3 Hz, 1H), 2.33 (d, J = 6.3 Hz, 1H, 3′-OH), 1.97 (m, 4H), 1.65-1.22 (m, 20H), 1.13 (s, 3H), 1.12 (s, 3H), 0.84 (m, 3H).

A THF solution (5 mL) of **17c** (259 mg, 0.76 mmol) was treated with an aqueous solution of NaOH (0.30 mL, 5N, 1.52 mmol). The reaction was heated overnight at 50°C. The reaction mixture was cooled to 0°C and acidified to pH = 3 using 5N HCl. The reaction was then extracted with EtOAc (75 mL). The organic layer was washed with water and brine. The organic layer thus obtained was dried and conc. *in vacuo* to afford 222 mg (90%) of the crude acid **17d**, which was used directly for side chain coupling reaction.

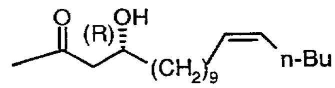
$^1$H NMR of **17d** ($CDCl_3$): $\delta$ 5.28 (m, 2H), 3.56 (m, 1H), 1.95 (m, 4H), 1.60-1.10 (m, 26H), 0.83 (m, 3H).

*Preparation of 17-1 and 17-2:*

R = 

$R^1$ = H

**17-1**

R = 

$R^1$ = H

**17-2**

A THF solution (7 mL) of the crude acid **17d** (240 mg, 0.74 mmol) was treated with HOBt (90.5 mg, 0.67 mmol) and EDCI (128 mg, 0.67 mmol) at rt. After stirring for 1.5 hr, DMAP (41 mg, 0.33 mmol) was added. After stirring for another 2 hr, a DMF (4 mL) of CBZ-protected psuedomycin nucleus (614 mg, 0.44 mmol) was added and the reaction was stirred at rt overnight. The reaction mixture was purified with preparative HPLC to give, after lyophilization, (160 mg, 21%) of the desired product.

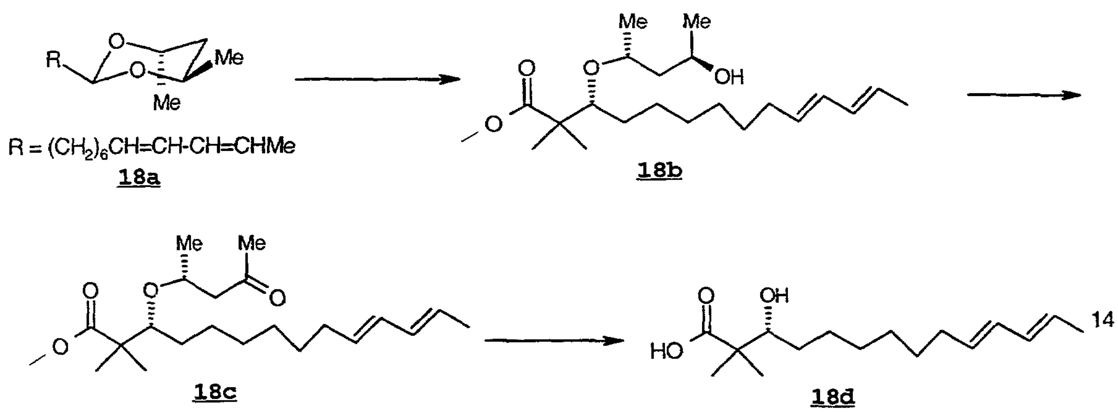
An acetonitrile solution (2 mL) of CBZ-protected compound (53.4 mg, 0.032 mmol) was treated with TMSI (77 mg, 0.38 mmol) at 0°C. After 100 min, the reaction was quenched with 1:1 $CH_3CN/H_2O$. The resulting reaction mixture was purified by reverse phase preparative HPLC to give 25.8 mg (65%) of the desired final product **17-1**.

Compound **17-2** is made using the same procedures described above using the appropriate starting materials where R* is a hydrogen.

## *Example 18*

Example 18 illustrates the attachment of a chiral alkenyl side-chain.

*Preparation of side chain precursor 18d:*




To a dichloromethane solution (25 mL) of **18a** (993 mg, 3.73 mmol) was added at -78°C methyl trimethylsilyl dimethylketene acetal (2.27 mL, 11.2 mmol) and neat $TiCl_4$ (0.49 mL, 4.48 mmol). After 2 hr, the reaction was quenched at -78°C with MeOH (5 mL). The reaction mixture was extracted with dichloromethane (3 x 40 mL). The combined organic layers were washed with $NaHCO_3$ and brine. The organic layer was dried and conc. *in vacuo* to yield a residue, which was purified with chromatography (15-20% EtOAc/Hexanes) to provide 837 mg (61%) of the desired product **18b**.

$^1$H NMR of **18a** ($CDCl_3$): δ 6.28-5.87 (m, 2H), 5.62-5.43 (m, 2H), 4.75 (m, 1H), 4.22 (m, 1H), 3.87 (m, 1H), 2.08-1.93 (m,

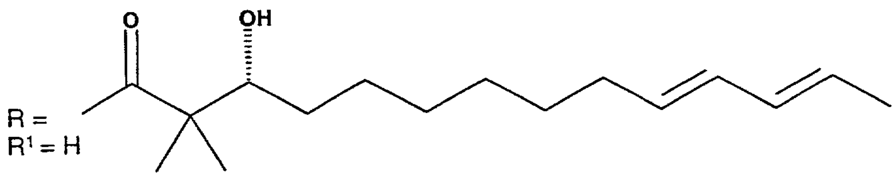
2H), 1.80-1.60 (m, 3H), 1.60-1.40 (m, 3H), 1.38-1.10 (m, 15H). $^1$H NMR of **18b** ($CDCl_3$): δ 5.98-5.90 (m, 2H), 5.55-5.47 (m, 2H), 4.08 (m, 1H), 3.86 (m, 1H), 3.61 (s, 3H), 3.50 (m, 1H), 2.00-1.96 (m, 2H), 1.74-1.63 (m, 3H), 1.50-1.00 (m, 24H).

To a dichloromethane solution (22 mL) of **18b** (837 mg, 2.27 mmol) was added PCC (0.98 g, 4.54 mmol). The reaction was stirred at rt for 18 hr, and then filtered through a pad of Celite. The filtrates were concentrated in vacuo to give a reddish residue, which was purified by silica gel chromatography (20% EtOAc/Hexanes) to give 441 mg (53%) of the desired methyl ketone **18c**.

$^1$H NMR of **18c** ($CDCl_3$): δ 5.92-5.87 (m, 2H), 5.48-5.43 (m, 2H), 3.88 (m, 1H), 3.56 (s, 3H), 3.49 (m, 1H), 2.59 (dd, J = 6.4, 14.7 Hz, 1H), 2.29 (dd, J = 5.9, 15.2 Hz, 1H), 2.06 (s, 3H), 1.96 (m, 2H), 1.63 (m, 2H), 1.40-0.90 (m, 20H).

To a THF (4 mL) and methane (2 mL) solution of **18c** (440 mg, 1.20 mmol) was added at rt 7.5M KOH (1 mL). After stirring at rt for 4 h, the reaction was acidified with 1N HCl to pH = 3. The reaction mixture was extracted with EtOAc (3 x 30 mL). The combined extracts were washed with water (2 x 10 mL) and brine. The organic layer was dried and conc. in vacuo to give 388 mg (>100%) of the crude acid **18d**, which was used directly for the side chain coupling reaction.

*Preparation of Compound 18-1:*



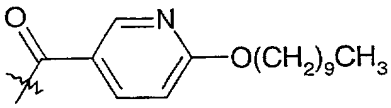
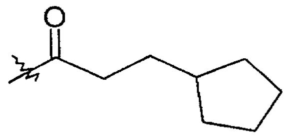
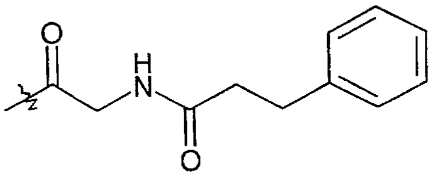
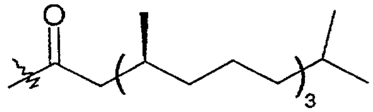
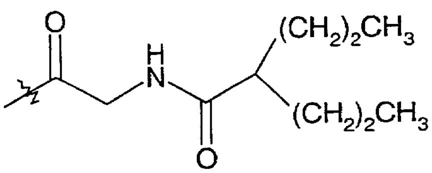
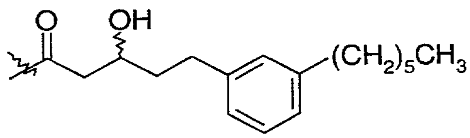
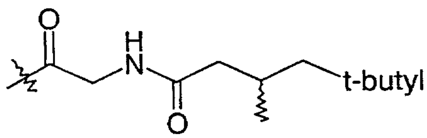
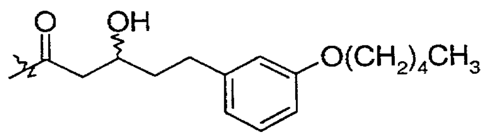
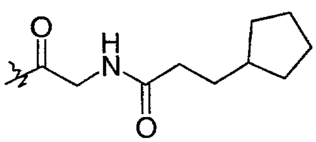
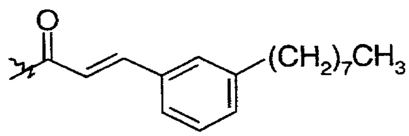
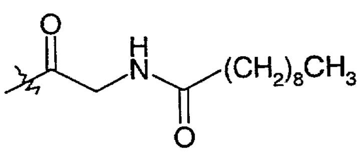
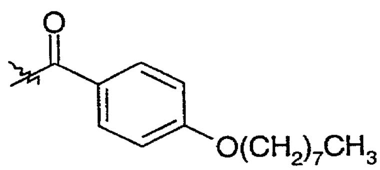
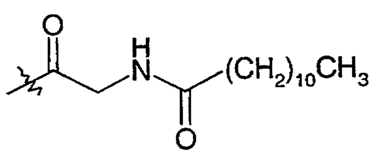
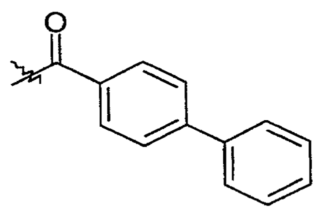
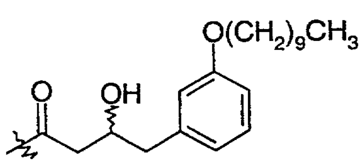
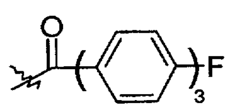
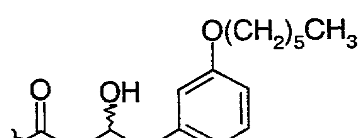
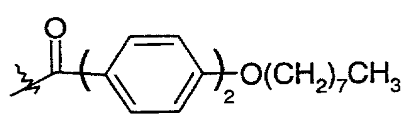

**18-1**

To a THF solution (10 mL) of the crude acid **18d** (~1.66 mmol) was added HOBt (244 mg, 1.66 mmol) and EDCI (318 mg, 1.66 mmol). After stirring at rt for 5 hr, a DMF solution (5 mL) of Alloc-protected pseudomycin nucleus (614 mg, 0.50 mmol) was added. After stirring at rt for a few days, DMAP (61 mg, 0.50 mmol) was added to the reaction mixture. After stirring for additional 12 hr, the reaction mixture was purified by reverse phase HPLC to afford, after lyophilization, 225 mg (30%) of alloc-protected acylated derivative.

To a degassed THF (20 mL) and HOAc (1 mL) solution of alloc-protected acylated derivative (240 mg, 0.16 mmol) was added $PdCl_2(PPh_3)_2$ (23 mg, 0.032 mmol) and $Bu_3SnH$ (0.87 mL, 3.23 mmol) at rt. After 1.5 hr, the reaction mixture was purified by preparative HPLC to afford 33 mg (17%) of Compound **18-1**.

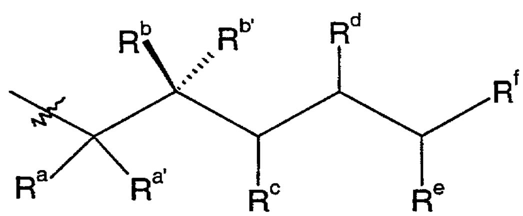
In addition to the compounds listed in the Examples above, the following N-acyl derivatives were also made which showed limited activity or non-significant activity.

Even though these compounds may not be as useful as antifungal agents, they provide valuable insight into the design of compounds having optimal activity.

Unless indicated otherwise, each of the compounds listed in the Examples showed measurable activity against *Candida Albicans*, *Cryptococcus neoformans*, *Aspergillus Fumigatus*, *Candida Parapsilosis*, or *Histoplasma capsulatum*. However, the following basic trends in activity were observed based on the compounds synthesized. The stereochemistry of the β-hydroxy group is preferably R. Longer alkyl chain lengths (i.e., $C_{12}$-$C_{20}$) tend to have higher activities than shorter alkyl chains (e.g., < $C_{11}$) regardless of stereochemistry or unsaturation levels. Removal of the β-hydroxy group, α,α-disubstitution, lower alkyl chain lengths in both alkyl and alkoxy substituents, extreme rigidity, and increased branching in the chain all tended to have lower activity than the longer flexible chains.

Consequently, alkyl side-chains represented by the following structure are preferred for antifungal treatment:




,

where

$R^a$ and $R^{a'}$ are independently hydrogen or methyl, or either $R^a$ or $R^{a'}$ is alkyl amino, taken together with $R^b$

or $R^{b'}$ forms a six-membered cycloalkyl ring, a six-membered aromatic ring or a double bond, or taken together with $R^c$ forms a six-membered aromatic ring;

$R^b$ and $R^{b'}$ are independently hydrogen or methyl, and either $R^b$ or $R^{b'}$ is hydroxy provided that $R^{b'}$ is not hydroxy when $R^a$, $R^b$, $R^d$, $R^e$ are hydrogen, $R^c$ is hydrogen and $R^f$ is *n*-hexyl, *n*-octyl or *n*-decyl, or $R^a$, $R^b$, $R^d$, $R^e$ are hydrogen, $R^c$ is hydroxy and $R^f$ is *n*-octyl, *n*-nonyl, or *n*-decyl;

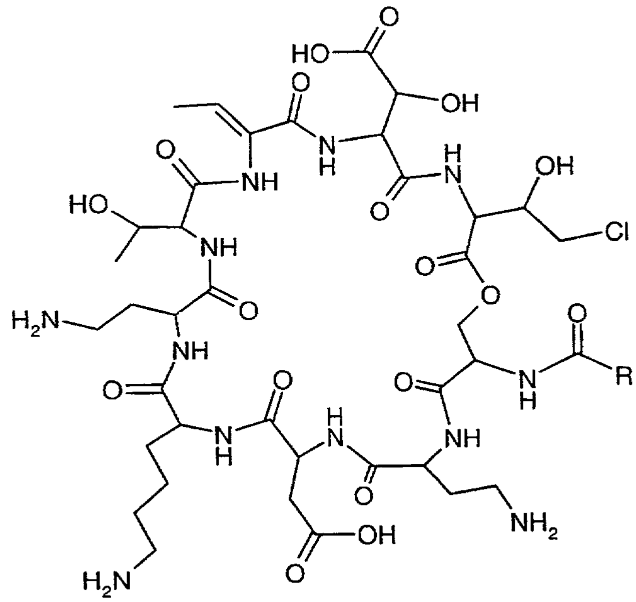
$R^c$ is hydrogen, hydroxy, $C_1$-$C_4$ alkoxy, hydroxyalkoxy, or taken together with $R^e$ forms a 6-membered aromatic ring or $C_5$-$C_6$ cycloalkyl ring;

$R^e$ is hydrogen, or taken together with $R^f$ is a six-membered aromatic ring, $C_5$-$C_{14}$ alkoxy substituted six-membered aromatic ring, or $C_5$-$C_{14}$ alkyl substituted six-membered aromatic ring, and

$R^f$ is $C_8$-$C_{14}$ alkyl, or $C_5$-$C_{11}$ alkoxy.

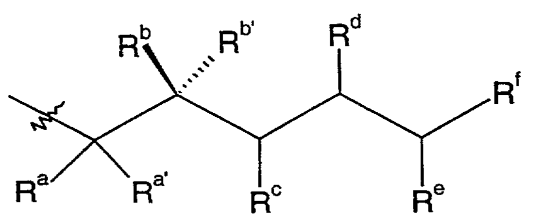
WE CLAIM:

1. A compound represented by structure I



I

wherein R is



where

$R^a$ and $R^{a'}$ are independently hydrogen or methyl, or either $R^a$ or $R^{a'}$ is alkyl amino, taken together with $R^b$ or $R^{b'}$ forms a six-membered cycloalkyl ring, a six-membered aromatic ring or a double bond, or taken together with $R^c$ forms a six-membered aromatic ring;

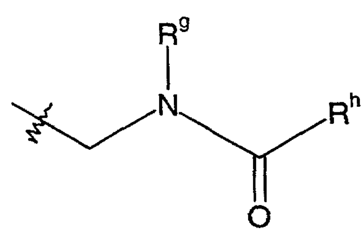
$R^b$ and $R^{b'}$ are independently hydrogen, halogen, or methyl, or either $R^b$ or $R^{b'}$ is amino, alkylamino, α-acetoacetate, methoxy, or hydroxy provided that $R^{b'}$ is not hydroxy when $R^a$, $R^b$, $R^d$, $R^e$ are hydrogen, $R^c$ is hydrogen and $R^f$ is *n*-hexyl, *n*-octyl or *n*-decyl, or $R^a$, $R^b$, $R^d$, $R^e$ are hydrogen, $R^c$ is hydroxy and $R^f$ is *n*-octyl, *n*-nonyl, or *n*-decyl;

$R^c$ is hydrogen, hydroxy, $C_1$-$C_4$ alkoxy, hydroxyalkoxy, or taken together with $R^e$ forms a 6-membered aromatic ring or $C_5$-$C_6$ cycloalkyl ring;

$R^e$ is hydrogen, or taken together with $R^f$ is a six-membered aromatic ring, $C_5$-$C_{14}$ alkoxy substituted six-membered aromatic ring, or $C_5$-$C_{14}$ alkyl substituted six-membered aromatic ring, and

$R^f$ is $C_8$-$C_{18}$ alkyl, $C_5$-$C_{11}$ alkoxy, or biphenyl; or

R is



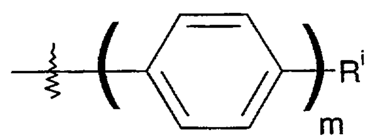

,

where

$R^g$ is hydrogen, or $C_1$-$C_{13}$ alkyl, and

$R^h$ is $C_1$-$C_{15}$ alkyl, $C_4$-$C_{15}$ alkoxy, ($C_1$-$C_{10}$ alkyl)phenyl, -$(CH_2)_n$-aryl, or -$(CH_2)_n$-($C_5$-$C_6$ cycloalkyl), where n = 1-2; or

R is



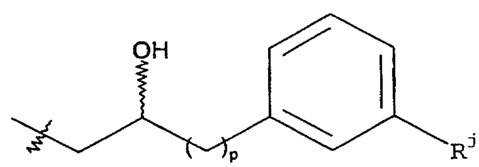
,

where

$R^i$ is a hydrogen, halogen, or $C_5$-$C_8$ alkoxy, and

m is 1, 2 or 3;

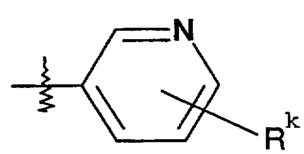
R is



where

$R^j$ is $C_5$-$C_{14}$ alkoxy or $C_5$-$C_{14}$ alkyl, and p = 0, 1 or 2;

R is



where

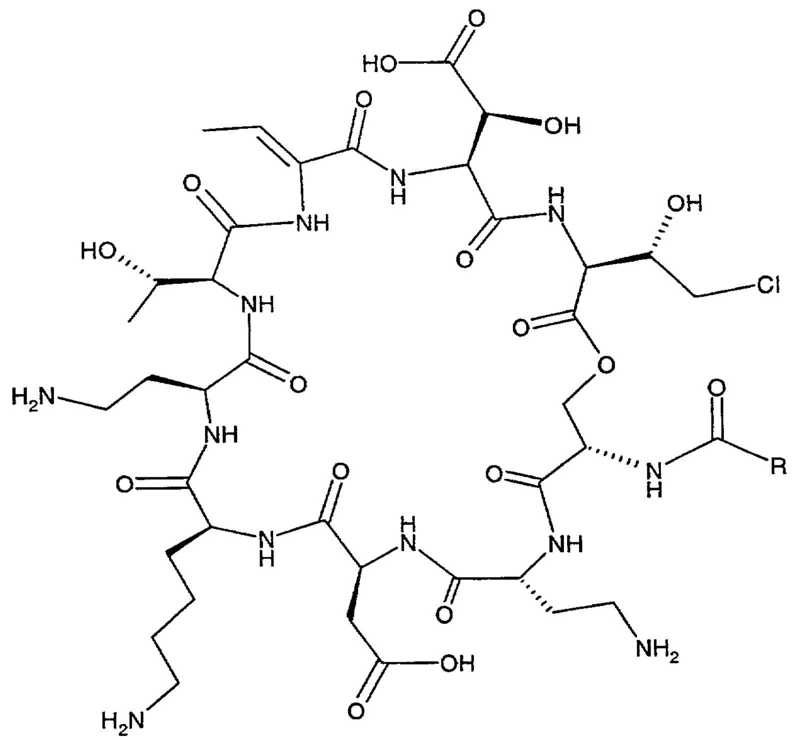
$R^k$ is $C_5$-$C_{14}$ alkoxy; or

R is -$(CH_2)$-$NR^m$-($C_{13}$-$C_{18}$ alkyl), where $R^m$ is H, -$CH_3$ or

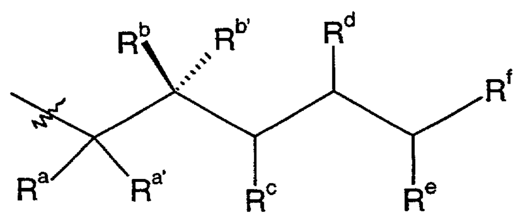
-C(O)$CH_3$; and

pharmaceutically acceptable salts and solvates thereof.

2. The compound of Claim 1 wherein structure I has the following stereochemistry



3. The compound of Claim 1 wherein R is



where

$R^a$ and $R^{a'}$ are independently hydrogen or methyl, or either $R^a$ or $R^{a'}$ is alkyl amino, taken together with $R^b$ or $R^{b'}$ forms a six-membered cycloalkyl ring, a six-membered aromatic ring or a double bond, or taken together with $R^c$ forms a six-membered aromatic ring;

$R^b$ and $R^{b'}$ are independently hydrogen, halogen, or methyl, or either $R^b$ or $R^{b'}$ is amino, alkylamino, α-acetoacetate, methoxy, or hydroxy provided that $R^{b'}$ is not hydroxy when $R^a$, $R^b$, $R^d$, $R^e$ are hydrogen, $R^c$ is hydrogen and $R^f$ is *n*-hexyl, *n*-octyl or *n*-decyl, or $R^a$, $R^b$, $R^d$, $R^e$ are hydrogen, $R^c$ is hydroxy and $R^f$ is *n*-octyl, *n*-nonyl, or *n*-decyl;

$R^c$ is hydrogen, hydroxy, $C_1$-$C_4$ alkoxy, hydroxyalkoxy, or taken together with $R^e$ forms a 6-membered aromatic ring or $C_5$-$C_6$ cycloalkyl ring;

$R^e$ is hydrogen, or taken together with $R^f$ is a six-membered aromatic ring, $C_5$-$C_{14}$ alkoxy substituted six-membered aromatic ring, or $C_5$-$C_{14}$ alkyl substituted six-membered aromatic ring, and

$R^f$ is $C_8$-$C_{18}$ alkyl, $C_5$-$C_{11}$ alkoxy, or biphenyl.

4. The compound of Claim 3 wherein $R^{b'}$ is hydroxy provided that $R^c$ is not hydrogen when $R^a$, $R^b$, $R^d$, $R^e$ are

hydrogen and $R^f$ is *n*-hexyl, *n*-octyl or *n*-decyl, or $R^c$ is not hydroxy when $R^f$ is *n*-octyl, *n*-nonyl, or *n*-decyl.

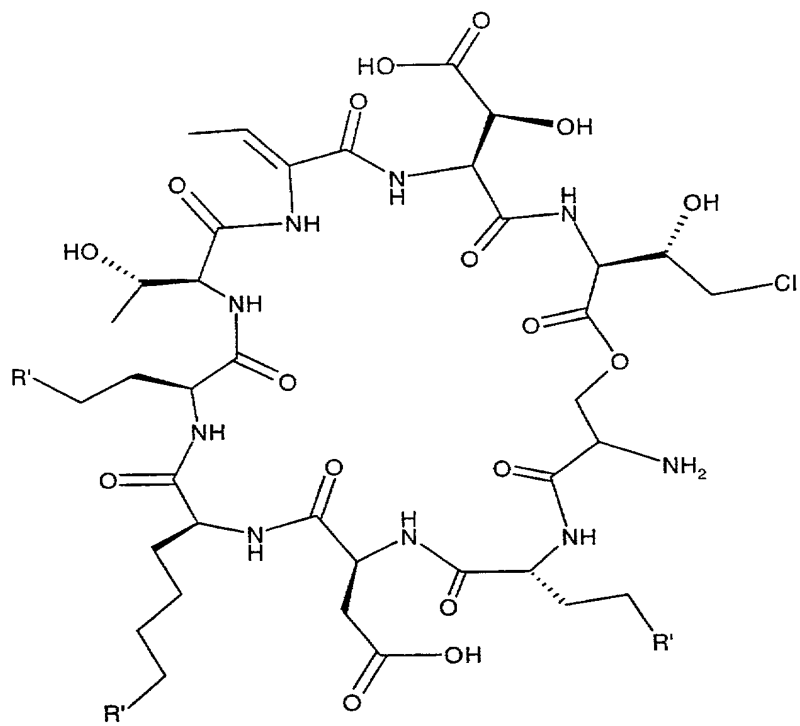
5. The use of a compound as claimed in any one of the preceding claims in the preparation of a medicament for use in combating either systemic fungal infections or fungal skin infections.

6. A pharmaceutical formulation comprising a pseudomycin compound of Claim 2 and a pharmaceutically acceptable carrier.

7. A method for treating an antifungal infection in an animal in need thereof, comprising the steps of administering to said animal a pseudomycin compound of Claim 2.

8. A process for producing a pseudomycin nucleus comprising the steps of providing a pseudomycin compound having an N-acyl alkyl side-chain containing at least one gamma or delta hydroxy group and reacting said pseudomycin compound with an acid to produce said pseudomycin nucleus.

9. The process of Claim 8 wherein said pseudomycin nucleus is represented by structure I-A



**I-A**

wherein R′ is $-NH_2$ or -NHp-Pg where Pg is an amino protecting group and p is 0 or 1.

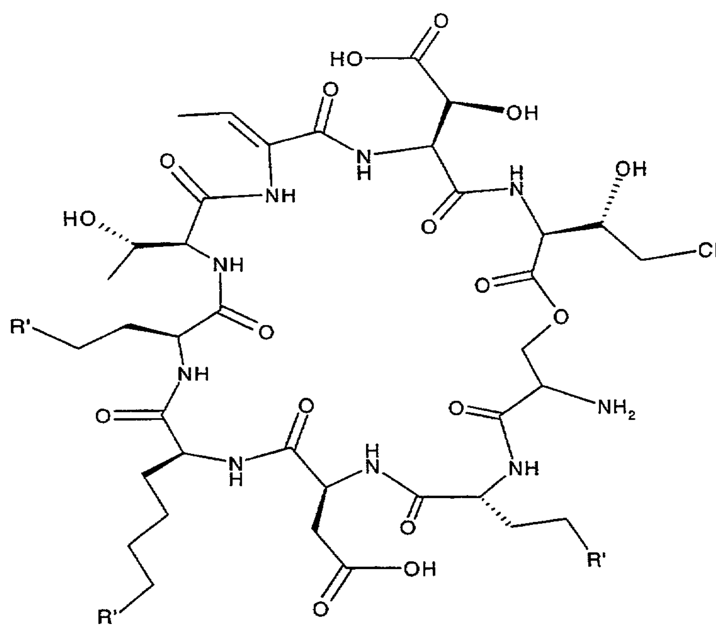
10. The process of Claim 8 wherein said pseudomycin compound having an N-acyl alkyl side-chain containing at least one gamma or delta hydroxy group is selected from the group consisting of pseudomycin A, pseudomycin A′ and pseudomycin C.

11. The process of Claim 8 wherein said acid is trifluoroacetic acid or acetic acid.

12. The process of Claim 11 wherein said acid is trifluoroacetic acid.

13. A pseudomycin nucleus prepared by the process of Claim 8.

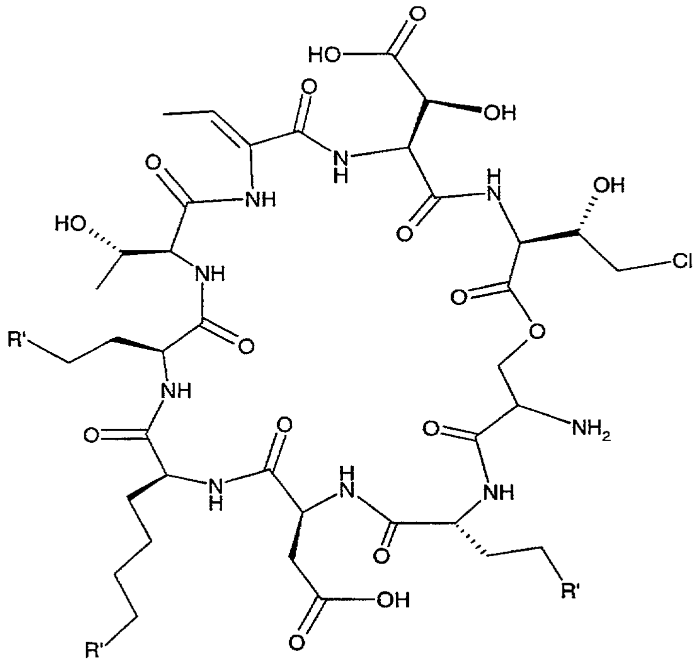
14. The pseudomycin nucleus of Claim 13 wherein said nucleus is represented by structure I-A




**I-A**

wherein R′ is $-NH_2$ or $-NH_p-Pg$ where Pg is an amino protecting group and p is 0 or 1.

15. A pseudomycin nucleus represented by structure I-A



**I-A**

wherein R′ is $-NH_2$ or -NHp-Pg where Pg is an amino protecting group and p is 0 or 1.

Please type a plus sign (+) inside this box [+]

PTO/SB/01 (8-96) (MODIFIED)

Approved for use through 9/30/98. OMB 0651-0032
Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

# DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION

| | |
|---|---|
| Attorney Docket Number | X-11260 |
| First Named Inventor | Matthew David Belvo |
| *COMPLETE IF KNOWN* | |
| Application Number | |
| Filing Date | |
| Group Art Unit | |
| Examiner Name | |

[X] Declaration Submitted with Initial Filing
[ ] Declaration Submitted after Initial Filing

**As a below named Inventor, I hereby declare that:**

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole Inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the Invention entitled:

**PSEUDOMYCIN N-ACYL SIDE-CHAIN ANALOGS**

the specification of which
[ ] is attached hereto
OR
[X] was filed on (MM/DD/YYYY) 08 June 2000 as United States Application Number or PCT International Application Number PCT/US00/15017 and was amended on (MM/DD/YYYY) [ ] (if applicable).

II hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37 Code of Federal Regulations, § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or Inventor's certificate, or § 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

| Prior Foreign Application Number(s) | Country | Foreign Filing Date (MM/DD/YYYY) | Priority Not Claimed | Certified Copy Attached YES | NO |
|---|---|---|---|---|---|
| | | | [ ] | [ ] | [ ] |
| | | | [ ] | [ ] | [ ] |
| | | | [ ] | [ ] | [ ] |
| | | | [ ] | [ ] | [ ] |
| | | | [ ] | [ ] | [ ] |
| | | | [ ] | [ ] | [ ] |

[ ] Additional foreign application numbers are listed on a supplemental priority sheet attached hereto:

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional applications(s) listed below.

| Application Number(s) | Filing Date (MM/DD/YYYY) | |
|---|---|---|
| 60/143,989 | 15 July 1999 | [ ] Additional provisional application numbers are listed on a supplemental priority sheet attached hereto. |

Please type a plus sign (+) inside this box [+]

PTO/SB/01 (8-96) (MODIFIED)

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Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

# DECLARATION

I hereby claim the benefit under Title 35, United States Code §120 of any United States application(s), or § 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of Title 35, United States Code § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

| U.S. Parent Application Number | PCT Parent Number | Parent Filing Date (MM/DD/YYYY) | Parent Patent Number *(if applicable)* |
|---|---|---|---|
| | | | |

☐ Additional U.S. or PCT international application numbers are listed on a supplemental priority sheet attached hereto.

As a named inventor, I hereby appoint the following registered practitioner(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

| Attorney Name | Reg. No. |
|---|---|
| Arvie J. Anderson | 45,263 |
| Lynn D. Apelgren | 45,341 |
| Robert A. Armitage | 27,417 |
| Brian P. Barrett | 39,597 |
| Michael T. Bates | 34,121 |
| Roger S. Benjamin | 27,025 |
| Gary M. Birch | 48,881 |
| William R. Boudreaux | 35,796 |
| Steven P. Caltrider | 36,467 |
| Paul R. Cantrell | 36,470 |
| Charles E. Cohen | 34,565 |
| Donald L. Comeglio | 30,741 |
| Gregory A. Cox | 47,504 |
| Paula K. Davis | 47,517 |
| Elizabeth A. Dawalt | 44,646 |
| John C. Demeter | 30,167 |
| Manisha A. Desai | 43,585 |
| Joanne Longo Feeney | 35,134 |
| Paul J. Gaylo | 36,808 |
| Francis O. Ginah | 44,712 |
| Janet A. Gongola | 48,436 |
| Amy E. Hamilton | 33,894 |
| Frederick D. Hunter | 26,915 |
| Thomas E. Jackson | 33,064 |
| Charles Joyner | 30,466 |
| Gerald P. Keleher | 43,707 |
| | |
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| Attorney Name | Reg. No. |
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| James J. Kelley | 41,888 |
| Paul J. Koivuniemi | 31,533 |
| Robert E. Lee | 27,919 |
| Kirby Lee | 47,744 |
| James P. Leeds | 35,241 |
| Nelsen L. Lentz | 38,537 |
| Douglas K. Norman | 33,267 |
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| Thomas G. Plant | 35,784 |
| Edward Prein | 37,212 |
| Grant E. Reed | 41,264 |
| James J. Sales | 33,773 |
| Michael J. Sayles | 32,295 |
| Robert L. Sharp | 45,609 |
| David M. Stemerick | 40,187 |
| Mark J. Stewart | 43,936 |
| Robert D. Titus | 40,206 |
| Robert C. Tucker | 45,165 |
| Tina M. Tucker | 47,145 |
| MaCharri Vorndran-Jones | 36,711 |
| Gilbert T. Voy | 43,972 |
| Thomas D. Webster | 39,872 |
| Lawrence T. Welch | 29,487 |
| Alexander Wilson | 45,782 |
| Dan L. Wood | 48,613 |
| | |
| | |

☐ Additional registered practitioner(s) named on a supplemental sheet attached hereto.

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| Name | ELI LILLY AND COMPANY | | | | |
| Address | ATTN: TINA M. TUCKER | | | | |
| Address | LILLY CORPORATE CENTER/DC1104 | | | | |
| City | INDIANAPOLIS | State | INDIANA | ZIP | 46285 |
| Country | | Telephone | (317) 277-3537 | Fax | (317) 276-3861 |

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1-

**Name of Sole or First Inventor:** ☐ **A Petition has been filed for this unsigned inventor**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Given Name | Matthew | Middle Name | David | Family Name | Belvo | Suffix e.g. Jr. | |
| Inventor's Signature | Matthew David Belvo | | | | | Date | 11-28-01 |
| Residence: City | Greenfield | State | IN | Country | USA | Citizenship | USA |
| Address | 24 Greenbrook Court IN | | | | | | |
| Post Office Address | SAME AS ABOVE | | | | | | |
| | | | | | | | |
| City | Greenfield | State | IN | Zip | 46140 | Country | USA |

[x] Additional Inventors are being named on supplement sheet(s) attached hereto.

Please type a plus sign (+) inside this box [+]

PTO/SB/01 (8-96) (MODIFIED)

Approved for use through 9/30/98. OMB 0651-0032
Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

# DECLARATION

2-00

| Name of Additional Joint Inventor, if any: | ☐ A Petition has been filed for this unsigned inventor | | | | | | |
|---|---|---|---|---|---|---|---|
| Given Name | Shu | Middle Name | Hui | Family Name | Chen | Suffix e.g. Jr. | |
| Inventor's Signature | Chen Shu Hui | | | | | Date | 11/30/2001 |
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3-00

| Name of Additional Joint Inventor, if any: | ☐ A Petition has been filed for this unsigned inventor | | | | | | |
|---|---|---|---|---|---|---|---|
| Given Name | Christopher | Middle Name | William | Family Name | Doecke | Suffix e.g. Jr. | |
| Inventor's Signature | Christopher William Doecke | | | | | Date | 12/7/2001 |
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| Post Office Address | SAME AS ABOVE | | | | | | |
| City | Indianapolis | State | IN | Zip | 46256 | Country | USA |

4-00

| Name of Additional Joint Inventor, if any: | ☐ A Petition has been filed for this unsigned inventor | | | | | | |
|---|---|---|---|---|---|---|---|
| Given Name | Sarah | Middle Name | Lynne | Family Name | Hellman | Suffix e.g. Jr. | |
| Inventor's Signature | Sarah Lynne Hellman | | | | | Date | 11/21/01 |
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| Post Office Address | SAME AS ABOVE | | | | | | |
| City | Indianapolis | State | IN | Zip | 46254 | Country | USA |

5-00

| Name of Additional Joint Inventor, if any: | ☐ A Petition has been filed for this unsigned inventor | | | | | | |
|---|---|---|---|---|---|---|---|
| Given Name | James | Middle Name | Andrew | Family Name | Jamison | Suffix e.g. Jr. | |
| Inventor's Signature | Jim Andrew Jamison | | | | | Date | 11/28/01 |
| Residence: City | Indianapolis | State | IN | Country | USA | Citizenship | USA |
| Post Office Address | 7009 Ringtail Court IN | | | | | | |
| Post Office Address | SAME AS ABOVE | | | | | | |
| City | Indianapolis | State | IN | Zip | 46254 | Country | USA |

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PTO/SB/01 (8-96) (MODIFIED)

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Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

# DECLARATION

| Name of Additional Joint Inventor, if any: | [ ] A Petition has been filed for this unsigned inventor | | | | | | |
|---|---|---|---|---|---|---|---|
| Given Name | Lawrence | Middle Name | Edward | Family Name | Patterson | Suffix e.g. Jr. | |
| Inventor's Signature | | | | | | Date | |
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| Post Office Address | SAME AS ABOVE | | | | | | |
| City | Indianapolis | State | IN | Zip | 46226 | Country | USA |

| Name of Additional Joint Inventor, if any: | [ ] A Petition has been filed for this unsigned inventor | | | | | | |
|---|---|---|---|---|---|---|---|
| Given Name | Michael | Middle Name | John | Family Name | Rodriguez | Suffix e.g. Jr. | |
| Inventor's Signature | | | | | | Date | |
| Residence: City | Indianapolis | State | IN | Country | USA | Citizenship | USA |
| Post Office Address | 7649 Gordonshire Court | | | | | | |
| Post Office Address | SAME AS ABOVE | | | | | | |
| City | Indianapolis | State | IN | Zip | 46278 | Country | USA |

| Name of Additional Joint Inventor, if any: | [ ] A Petition has been filed for this unsigned inventor | | | | | | |
|---|---|---|---|---|---|---|---|
| Given Name | Xicheng | Middle Name | David | Family Name | Sun | Suffix e.g. Jr. | |
| Inventor's Signature | [signature] | | | | | Date | 11/30/2001 |
| Residence: City | ~~Superior~~ | State | CO | Country | USA | Citizenship | China |
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| City | Superior | State | CO | Zip | 80027 | Country | USA |

| Name of Additional Joint Inventor, if any: | [ ] A Petition has been filed for this unsigned inventor | | | | | | |
|---|---|---|---|---|---|---|---|
| Given Name | William | Middle Name | Wilson | Family Name | Turner | Suffix e.g. Jr. | |
| Inventor's Signature | | | | | | Date | |
| Residence: City | Bloomington | State | IN | Country | USA | Citizenship | USA |
| Post Office Address | 4000 Saratoga Drive | | | | | | |
| Post Office Address | SAME AS ABOVE | | | | | | |
| City | Bloomington | State | IN | Zip | 47408 | Country | USA |

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Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

# DECLARATION

| Name of Additional Joint Inventor, if any: | | [ ] A Petition has been filed for this unsigned inventor | | | | | |
|---|---|---|---|---|---|---|---|
| Given Name | Lawrence | Middle Name | Edward | Family Name | Patterson | Suffix e.g. Jr. | |
| Inventor's Signature | Lawrence Edward Patterson | | | | Date | 11-28-01 | |
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| Address | 3839 East 42nd Street IN | | | | | | |
| Post Office Address | SAME AS ABOVE | | | | | | |
| City | Indianapolis | State | IN | Zip | 46226 | Country | USA |

6-00

| Name of Additional Joint Inventor, if any: | | [ ] A Petition has been filed for this unsigned inventor | | | | | |
|---|---|---|---|---|---|---|---|
| Given Name | Michael | Middle Name | John | Family Name | Rodriguez | Suffix e.g. Jr. | |
| Inventor's Signature | Michael John Rodriguez | | | | Date | 11/29/01 | |
| Residence: City | ~~Indianapolis~~ | State | IN | Country | USA | Citizenship | USA |
| Post Office Address | 7649 Gordonshire Court IN | | | | | | |
| Post Office Address | SAME AS ABOVE | | | | | | |
| City | Indianapolis | State | IN | Zip | 46278 | Country | USA |

7-00

| Name of Additional Joint Inventor, if any: | | [ ] A Petition has been filed for this unsigned inventor | | | | | |
|---|---|---|---|---|---|---|---|
| Given Name | Xicheng | Middle Name | David | Family Name | Sun | Suffix e.g. Jr. | |
| Inventor's Signature | | | | | Date | | |
| Residence: City | Superior | State | CO | Country | USA | Citizenship | China |
| Post Office Address | 923 Grays Peak Drive | | | | | | |
| Post Office Address | SAME AS ABOVE | | | | | | |
| City | Superior | State | CO | Zip | 80027 | Country | USA |

| Name of Additional Joint Inventor, if any: | | [ ] A Petition has been filed for this unsigned inventor | | | | | |
|---|---|---|---|---|---|---|---|
| Given Name | William | Middle Name | Wilson | Family Name | Turner | Suffix e.g. Jr. | |
| Inventor's Signature | William Wilson Turner | | | | Date | 11/29/01 | |
| Residence: City | ~~Bloomington~~ | State | IN | Country | USA | Citizenship | USA |
| Post Office Address | 4000 Saratoga Drive IN | | | | | | |
| Post Office Address | SAME AS ABOVE | | | | | | |
| City | Bloomington | State | IN | Zip | 47408 | Country | USA |

9-00

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PTO/SB/01 (8-96) (MODIFIED)

Approved for use through 9/30/98. OMB 0651-0032
Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

# DECLARATION

| Name of Additional Joint Inventor, if any: | | | | ☐ A Petition has been filed for this unsigned inventor | | | |
|---|---|---|---|---|---|---|---|
| Given Name | Venkatraghavan | Middle Name | | Family Name | Vasudevan | Suffix e.g. Jr. | |
| Inventor's Signature | Venkatraghavan Vasudevan | | | | | Date | 12-12-01 |
| Residence: City | Indianapolis | State | IN | Country | USA IN | Citizenship | USA |
| Address | 1016 Saratoga Circle | | | | | | |
| Post Office Address | SAME AS ABOVE | | | | | | |
| City | Indianapolis | State | IN | Zip | 46280 | Country | USA |

| Name of Additional Joint Inventor, if any: | | | | ☐ A Petition has been filed for this unsigned inventor | | | |
|---|---|---|---|---|---|---|---|
| Given Name | Mark | Middle Name | James | Family Name | Zweifel | Suffix e.g. Jr. | |
| Inventor's Signature | Mark James Zweifel | | | | | Date | 11-28-01 |
| Residence: City | Mooresville | State | IN | Country | USA IN | Citizenship | USA |
| Post Office Address | 1840 Centenary Road | | | | | | |
| Post Office Address | SAME AS ABOVE | | | | | | |
| City | Mooresville | State | IN | Zip | 46158 | Country | USA |

| Name of Additional Joint Inventor, if any: | | | | ☐ A Petition has been filed for this unsigned inventor | | | |
|---|---|---|---|---|---|---|---|
| Given Name | | Middle Name | | Family Name | | Suffix e.g. Jr. | |
| Inventor's Signature | | | | | | Date | |
| Residence: City | | State | | Country | | Citizenship | |
| Post Office Address | | | | | | | |
| Post Office Address | SAME AS ABOVE | | | | | | |
| City | | State | | Zip | | Country | |

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|---|---|---|---|---|---|---|---|
| Given Name | | Middle Name | | Family Name | | Suffix e.g. Jr. | |
| Inventor's Signature | | | | | | Date | |
| Residence: City | | State | | Country | | Citizenship | |
| Post Office Address | | | | | | | |
| Post Office Address | SAME AS ABOVE | | | | | | |
| City | | State | | Zip | | Country | |

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PTO/SB/01 (8-96) (MODIFIED)

Approved for use through 9/30/98. OMB 0651-0032
Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

# DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION

| | |
|---|---|
| **Attorney Docket Number** | **X-11260** |
| **First Named Inventor** | **Matthew David Belvo** |
| ***COMPLETE IF KNOWN*** | |
| **Application Number** | |
| **Filing Date** | |
| **Group Art Unit** | |
| **Examiner Name** | |

[X] Declaration Submitted with Initial Filing
[ ] Declaration Submitted after Initial Filing

**As a below named Inventor, I hereby declare that:**

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole Inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the Invention entitled:

**PSEUDOMYCIN N-ACYL SIDE-CHAIN ANALOGS**

the specification of which
[ ] is attached hereto
OR
[X] was filed on (MM/DD/YYYY) 08 June 2000 as United States Application Number or PCT International

Application Number PCT/US00/15017 and was amended on (MM/DD/YYYY) ____ (if applicable).

II hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment specifically referred to above.

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| Prior Foreign Application Number(s) | Country | Foreign Filing Date (MM/DD/YYYY) | Priority Not Claimed | Certified Copy Attached YES | NO |
|---|---|---|---|---|---|
| | | | [ ] | [ ] | [ ] |
| | | | [ ] | [ ] | [ ] |
| | | | [ ] | [ ] | [ ] |
| | | | [ ] | [ ] | [ ] |
| | | | [ ] | [ ] | [ ] |
| | | | [ ] | [ ] | [ ] |

[ ] Additional foreign application numbers are listed on a supplemental priority sheet attached hereto:

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional applications(s) listed below.

| Application Number(s) | Filing Date (MM/DD/YYYY) | |
|---|---|---|
| 60/143,989 | 15 July 1999 | [ ] Additional provisional application numbers are listed on a supplemental priority sheet attached hereto. |

Please type a plus sign (+) inside this box [+]

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| U.S. Parent Application Number | PCT Parent Number | Parent Filing Date (MM/DD/YYYY) | Parent Patent Number *(if applicable)* |
|---|---|---|---|
| | | | |

☐ Additional U.S. or PCT international application numbers are listed on a supplemental priority sheet attached hereto.

As a named inventor, I hereby appoint the following registered practitioner(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

| Attorney Name | Reg. No. |
|---|---|
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| Lynn D. Apelgren | 45,341 |
| Robert A. Armitage | 27,417 |
| Brian P. Barrett | 39,597 |
| Michael T. Bates | 34,121 |
| Roger S. Benjamin | 27,025 |
| Gary M. Birch | 48,881 |
| William R. Boudreaux | 35,796 |
| Steven P. Caltrider | 36,467 |
| Paul R. Cantrell | 36,470 |
| Charles E. Cohen | 34,565 |
| Donald L. Corneglio | 30,741 |
| Gregory A. Cox | 47,504 |
| Paula K. Davis | 47,517 |
| Elizabeth A. Dawalt | 44,646 |
| John C. Demeter | 30,167 |
| Manisha A. Desai | 43,585 |
| Joanne Longo Feeney | 35,134 |
| Paul J. Gaylo | 36,808 |
| Francis O. Ginah | 44,712 |
| Janet A. Gongola | 48,436 |
| Amy E. Hamilton | 33,894 |
| Frederick D. Hunter | 26,915 |
| Thomas E. Jackson | 33,064 |
| Charles Joyner | 30,466 |
| Gerald P. Keleher | 43,707 |

| Attorney Name | Reg. No. |
|---|---|
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| Paul J. Koivuniemi | 31,533 |
| Robert E. Lee | 27,919 |
| Kirby Lee | 47,744 |
| James P. Leeds | 35,241 |
| Nelsen L. Lentz | 38,537 |
| Douglas K. Norman | 33,267 |
| Arleen Palmberg | 40,422 |
| Thomas G. Plant | 35,784 |
| Edward Prein | 37,212 |
| Grant E. Reed | 41,264 |
| James J. Sales | 33,773 |
| Michael J. Sayles | 32,295 |
| Robert L. Sharp | 45,609 |
| David M. Stemerick | 40,187 |
| Mark J. Stewart | 43,936 |
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| Tina M. Tucker | 47,145 |
| MaCharri Vorndran-Jones | 36,711 |
| Gilbert T. Voy | 43,972 |
| Thomas D. Webster | 39,872 |
| Lawrence T. Welch | 29,487 |
| Alexander Wilson | 45,782 |
| Dan L. Wood | 48,613 |

☐ Additional registered practitioner(s) named on a supplemental sheet attached hereto.

Direct all correspondence to:

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|---|---|---|---|---|---|
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| Address | ATTN: TINA M. TUCKER | | | | |
| Address | LILLY CORPORATE CENTER/DC1104 | | | | |
| City | INDIANAPOLIS | State | INDIANA | ZIP | 46285 |
| Country | | Telephone | (317) 277-3537 | Fax | (317) 276-3861 |

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

**Name of Sole or First Inventor:** ☐ **A Petition has been filed for this unsigned inventor**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Given Name | Matthew | Middle Name | David | Family Name | Belvo | Suffix e.g. Jr. | |
| Inventor's Signature | | | | Date | | | |
| Residence: City | Greenfield | State | IN | Country | USA | Citizenship | USA |
| Address | 24 Greenbrook Court | | | | | | |
| Post Office Address | SAME AS ABOVE | | | | | | |
| City | Greenfield | State | IN | Zip | 46140 | Country | USA |

[x] Additional Inventors are being named on supplement sheet(s) attached hereto.

Please type a plus sign (+) inside this box [+]

PTO/SB/01 (8-96) (MODIFIED)

Approved for use through 9/30/98. OMB 0651-0032
Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

# DECLARATION

| Name of Additional Joint Inventor, if any: | | ☐ A Petition has been filed for this unsigned inventor | | | | | |
|---|---|---|---|---|---|---|---|
| Given Name | Shu | Middle Name | Hui | Family Name | Chen | Suffix e.g. Jr. | |
| Inventor's Signature | | | | | | Date | |
| Residence: City | Carmel | State | IN | Country | USA | Citizenship | USA |
| Address | 13256 Snow Owl Drive | | | | | | |
| Post Office Address | SAME AS ABOVE | | | | | | |
| City | Carmel | State | IN | Zip | 46033 | Country | USA |

| Name of Additional Joint Inventor, if any: | | ☐ A Petition has been filed for this unsigned inventor | | | | | |
|---|---|---|---|---|---|---|---|
| Given Name | Christopher | Middle Name | William | Family Name | Doecke | Suffix e.g. Jr. | |
| Inventor's Signature | | | | | | Date | |
| Residence: City | Indianapolis | State | IN | Country | USA | Citizenship | USA |
| Post Office Address | 7515 East 80th Street | | | | | | |
| Post Office Address | SAME AS ABOVE | | | | | | |
| City | Indianapolis | State | IN | Zip | 46256 | Country | USA |

| Name of Additional Joint Inventor, if any: | | ☐ A Petition has been filed for this unsigned inventor | | | | | |
|---|---|---|---|---|---|---|---|
| Given Name | Sarah | Middle Name | Lynne | Family Name | Hellman | Suffix e.g. Jr. | |
| Inventor's Signature | | | | | | Date | |
| Residence: City | Indianapolis | State | IN | Country | USA | Citizenship | USA |
| Post Office Address | 7009 Ringtail Court | | | | | | |
| Post Office Address | SAME AS ABOVE | | | | | | |
| City | Indianapolis | State | IN | Zip | 46254 | Country | USA |

| Name of Additional Joint Inventor, if any: | | ☐ A Petition has been filed for this unsigned inventor | | | | | |
|---|---|---|---|---|---|---|---|
| Given Name | James | Middle Name | Andrew | Family Name | Jamison | Suffix e.g. Jr. | |
| Inventor's Signature | | | | | | Date | |
| Residence: City | Indianapolis | State | IN | Country | USA | Citizenship | USA |
| Post Office Address | 7009 Ringtail Court | | | | | | |
| Post Office Address | SAME AS ABOVE | | | | | | |
| City | Indianapolis | State | IN | Zip | 46254 | Country | USA |

Please type a plus sign (+) inside this box [+]

PTO/SB/01 (8-96) (MODIFIED)

Approved for use through 9/30/98. OMB 0651-0032
Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

# DECLARATION

| Name of Additional Joint Inventor, if any: | | ☐ A Petition has been filed for this unsigned inventor | | | | | |
|---|---|---|---|---|---|---|---|
| Given Name | Venkatraghavan | Middle Name | | Family Name | Vasudevan | Suffix e.g. Jr. | |
| Inventor's Signature | | | | | | Date | |
| Residence: City | Indianapolis | State | IN | Country | USA | Citizenship | USA |
| Address | 1016 Saratoga Circle | | | | | | |
| Post Office Address | SAME AS ABOVE | | | | | | |
| City | Indianapolis | State | IN | Zip | 46280 | Country | USA |

| Name of Additional Joint Inventor, if any: | | ☐ A Petition has been filed for this unsigned inventor | | | | | |
|---|---|---|---|---|---|---|---|
| Given Name | Mark | Middle Name | James | Family Name | Zweifel | Suffix e.g. Jr. | |
| Inventor's Signature | | | | | | Date | |
| Residence: City | Mooresville | State | IN | Country | USA | Citizenship | USA |
| Post Office Address | 1840 Centenary Road | | | | | | |
| Post Office Address | SAME AS ABOVE | | | | | | |
| City | Mooresville | State | IN | Zip | 46158 | Country | USA |

| Name of Additional Joint Inventor, if any: | | ☐ A Petition has been filed for this unsigned inventor | | | | | |
|---|---|---|---|---|---|---|---|
| Given Name | | Middle Name | | Family Name | | Suffix e.g. Jr. | |
| Inventor's Signature | | | | | | Date | |
| Residence: City | | State | | Country | | Citizenship | |
| Post Office Address | | | | | | | |
| Post Office Address | SAME AS ABOVE | | | | | | |
| City | | State | | Zip | | Country | |

| Name of Additional Joint Inventor, if any: | | ☐ A Petition has been filed for this unsigned inventor | | | | | |
|---|---|---|---|---|---|---|---|
| Given Name | | Middle Name | | Family Name | | Suffix e.g. Jr. | |
| Inventor's Signature | | | | | | Date | |
| Residence: City | | State | | Country | | Citizenship | |
| Post Office Address | | | | | | | |
| Post Office Address | SAME AS ABOVE | | | | | | |
| City | | State | | Zip | | Country | |